Transactions of the American Fisheries Society 144:1301–1310, 2015

ISSN: 0002-8487 print / 1548-8659 online
DOI: 10.1080/00028487.2015.1069212

ARTICLE

Identification and Distribution of Morphologically Conserved Smoothhound Sharks in the Northern Gulf of Mexico

Melissa M. Giresi
Department of Biology, Texas A&M University, 3258 TAMUS, College Station, Texas 77843, USA

R. Dean Grubbs
Florida State University Coastal and Marine Laboratory, 3618 Highway 98, St. Teresa, Florida 32358, USA

David S. Portnoy
Marine Genomics Laboratory, Harte Research Institute, Department of Life Sciences, Texas A&M University–Corpus Christi, 6300 Ocean Drive, Corpus Christi, Texas 78412, USA

William B. Driggers III and Lisa Jones
National Marine Fisheries Service, Southeast Fisheries Science Center, Mississippi Laboratories, Post Office Drawer 1207, Pascagoula, Mississippi 39568, USA

John R. Gold*
Marine Genomics Laboratory, Harte Research Institute, Department of Life Sciences, Texas A&M University–Corpus Christi, 6300 Ocean Drive, Corpus Christi, Texas 78412, USA

Abstract

Identification of sharks within the genus *Mustelus* (smoothhound sharks) is problematic because of extensive overlap in external morphology among species. Consequently, species-specific management of smoothhound shark resources is difficult when multiple species inhabit the same geographic region. The species identification and distribution of smoothhound sharks in the northern Gulf of Mexico (the Gulf) were assessed using sequences of mitochondrial DNA, nuclear-encoded microsatellites, and catch data. Phylogenetic analysis of 1,047 base pairs of mitochondrially encoded ND-2 sequences and Bayesian clustering of multilocus genotypes at 15 microsatellites revealed three genetically distinct monophyletic lineages (clades) of smoothhound sharks in the Gulf. Examination of external morphology revealed characters that distinguished each genetically distinct clade, and based on species descriptions and comparisons with the type and other specimens in established collections, the lineages were identified as Smooth Dogfish *Mustelus canis*, Florida Smoothhound *Mustelus norrisi*, and Gulf Smoothhound *Mustelus sinusmexicanus*. Two hundred and eighty-seven smoothhound sharks sampled from across the Gulf were then assigned unequivocally, based on genetic data, to one of the three species. Multifactorial analysis and homogeneity tests of species-specific means versus grand means of spatiotemporal factors (depth, longitude, and month) at capture indicated significant differences among the three species with respect to all three factors. On average, the Smooth Dogfish is found in deeper waters than the Gulf Smoothhound, whereas the Florida Smoothhound inhabits relatively shallow waters. A diagnostic key for the field identification of adult specimens of each species is provided.

*Corresponding author: goldfish@tamucc.edu
Received April 1, 2015; accepted June 30, 2015

Global expansion of commercial and recreational shark fisheries over the last several decades has prompted concerns over the sustainability and survival of both target and bycatch species (Compagno and Cook 1995; Stevens et al. 2000). Numerous fisheries targeting sharks have collapsed within decades of their inception (Musick et al. 2000; Campana et al. 2008; Chabot and Allen 2009), and when sharks are managed in mixed-species fisheries species-specific data go unrecorded, obscuring patterns of spatial and temporal catch rates for individual species. Because the more productive species in a mixed-species fishery sustain higher rates of fishing mortality than species with lower intrinsic rates of increase, the latter (especially if they are cryptic) are highly susceptible to population collapse and local extirpation (Musick 1999; Dulvy et al. 2000). Historically, several groups of sharks in U.S. waters have been managed as multispecies complexes, in large part because the conserved morphology of many species presents problems in field identification. The current trend in U.S. waters, however, is toward single-species management because of the susceptibility in mixed-species fisheries of individual species with relatively low productivity (Musick et al. 2000).

The triakid shark genus *Mustelus* (smoothhound sharks) contains 29 nominal species worldwide and is highly conserved in external morphology (Compagno et al. 2005; White and Last 2008). Globally, smoothhound sharks constitute important regional fisheries resources (Compagno et al. 2005; Castro 2011), and a number of species are listed as vulnerable, near-threatened, or endangered (IUCN 2013). The average annual landings (commercial and recreational) of smoothhound sharks in U.S. waters of the western Atlantic Ocean (hereafter, the Atlantic) between 1991 and 2012 was 1,059 tons (Cortés and Balchowsky 2014), making this one of the largest shark fisheries in U.S. waters (NMFS 2010a). The ongoing assessment of smoothhound sharks in the Gulf of Mexico (hereafter, the Gulf) (SEDAR 2015) is considered data poor or data limited because of the inability to discern among the three (possibly four) nominal smoothhound shark species reported to occur there (NMFS 2010a, 2010b).

The four nominal species (Smooth Dogfish *Mustelus canis*, Florida Smoothhound *Mustelus norrisi*, Gulf Smoothhound *Mustelus sinusmexicanus*, and Smalleye Smoothhound *Mustelus higmani*) are frequently misidentified due to the lack of clear and consistent external morphological characters that can be used to distinguish among them (Heemstra 1997; Compagno et al. 2005). The Smooth Dogfish is the most widely distributed of the four species, ranging from Massachusetts to northern Brazil (including the Gulf) and from southern Brazil through Argentina (Compagno et al. 2005). The Florida Smoothhound has a more limited range and is reported to occur from the northern Gulf to Brazil (Heemstra 1997; Compagno et al. 2005); The Gulf Smoothhound is thought to be endemic and restricted to the Gulf (Compagno et al. 2005). The fourth species, the Smalleye Smoothhound, was originally described (Springer and Lowe 1963) from Suriname and is known to occur primarily along the Atlantic coast of South America from Curaçao to Santos on the southern coast of Brazil (Heemstra 1997). A single specimen identified as a Smalleye Smoothhound was collected in the northeastern Gulf at a depth of more than 1,280 m, at least 400 m deeper than any previously recorded catches or sightings of a species of *Mustelus* (Heemstra 1997). Distributional data for Florida Smoothhounds, Gulf Smoothhounds, and Smalleye Smoothhounds are fairly limited, and the designation of Florida Smoothhounds has been questioned (NMFS 2010a, 2010b). Because reliable and consistent methods for distinguishing among these species of *Mustelus* in the field are unavailable, smoothhound sharks in U.S. waters of the Atlantic and Gulf are currently managed as a single, multispecies complex (NMFS 2010a, 2010b).

Studies by Heemstra (1997) indicated that the Florida Smoothhound matures at smaller sizes than either the Smooth Dogfish or the Gulf Smoothhound, and it is possible that other life history characteristics (e.g., age at maturity, maximum age, and fecundity) also differ among the species. If life history parameters do vary among the species, the intrinsic rate of population increase also may differ, meaning that each species could respond differently to fishing mortality. Consequently, the unequivocal identification, stock status, and distribution of each smoothhound shark species in U.S. waters are needed for effective conservation and management of these resources.

We assessed patterns of genetic divergence among smoothhound sharks sampled from U.S. waters of the Atlantic and Gulf, using sequences of mitochondrial DNA (mtDNA) and nuclear-encoded microsatellites to assess whether distinct genetic lineages (putative species) were present. We then made detailed comparisons of external morphology for a subset of specimens from the genetically distinct groups and identified each group to species by comparing the specimens with the type and other material in two different collections. In the process we developed a dichotomous key to distinguish among three of the species in the field, and we used temporal and spatial catch data to determine whether there were predictive variables of species presence/absence across the Gulf.

METHODS

A total of 287 adult smoothhound sharks were sampled from the Gulf (Figure 1) during bottom longline, trawl, and gill-net surveys carried out between 2010 and 2013 by personnel from the Coastal and Marine Laboratory of Florida State University (CML), the Mississippi laboratories of the Southeast Fisheries Science Center (National Marine Fisheries Service, National Oceanic and Atmospheric Administration [NMFS–NOAA]), the Texas Parks and Wildlife Department, and the Dauphin Island Sea Laboratory. Due to differences in depth and target taxa, gears and survey designs varied and most surveys did not sample in winter. However, depths from the shoreline to the lower continental slope, encompassing all

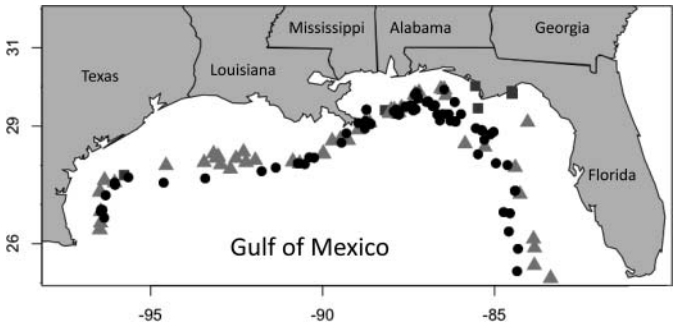


FIGURE 1. Locations from which smoothhound shark specimens were obtained in the northern Gulf of Mexico: circles = Smooth Dogfish, squares = Florida Smoothhound, and triangles = Gulf Smoothhound. A number of specimens were sampled from each locality (see Table S.1).

of the depths at which species of *Mustelus* might occur, were sampled. For example, the CML GulfSPAN Survey (longline and gill-net) sampled depths of 1–7 m (12 months/year), the NMFS–NOAA) Groundfish Survey (trawl) sampled depths of 9–110 m (summer–fall), the NMFS–NOAA Longline Survey sampled depths of 9–183 m (summer), and the CML Deep-C Survey (longline and trap) sampled depths of 171–2,000 m (spring, summer, and fall). A single specimen of Smooth Dogfish, sampled near Cape Cod Bay, Massachusetts, was provided by the Massachusetts Division of Marine Fisheries. Most (264) of the individuals sampled were tentatively identified to species in the field. A list of individuals sampled by year and month of capture, locality, and depth may be found in Supplementary Table S.1 available in the online version of this article. Fin clips ($\sim$1 cm^2) were taken either from the trailing edge of the first dorsal fin, the left pelvic fin, or the subterminal notch of the caudal fin and fixed in either 20% dimethyl sulfoxide storage buffer (Seutin et al. 1991) or 95% ethanol. Fin clips from 10 smoothhound sharks identified in the field as Smalleye Smoothhounds were obtained by NOAA personnel from offshore of French Guiana. Whole genomic DNA was extracted using a modified Chelex extraction protocol (Estoup et al. 1996). A total of 46 whole smoothhound specimens (45 from the Gulf and the specimen of Smooth Dogfish from near Cape Cod Bay) were set aside for examination of external morphology.

A 1,047 base-pair (bp) fragment of the mitochondrial gene encoding the NADH-dehydrogenase subunit-2 gene (*ND-2*) was amplified from a subset of 132 individuals. Polymerase chain reaction (PCR) primers *Mus*ND2F (5′-CCA TAC CCC AAC CAT GTG GTT-3′) and *Mus*ND2R (5′-GCT TTG AAG GCT TTT GGT CTG-3′) were designed based on conserved regions flanking the *ND-2* gene among 10 smoothhound species sequenced by Lopez et al. (2006). Thirty-microliter reactions contained 100 ng DNA, 1× PCR buffer, 0.5 U *Taq* DNA polymerase (GoTaq Flexi DNA Polymerase, Promega), 1.5 μM of each primer, 2.4 mM dNTPs, and 2.4 mM $MgCl_2$. The PCR amplification profile was as follows: initial denaturation at 95°C for 3 min; 40 cycles of 95°C for 30 s, 60°C for 1 min, and 72°C for 1 min; and final extension at 72°C for 10 min. Amplicons were electrophoresed on 2.0% agarose gels and extracted and purified using a QIAquick Gel Extraction Kit (Qiagen; www.qiagen.com). The PCR products were sequenced either at the Interdisciplinary Center for Biotechnology Research at the University of Florida (http://www.biotech.ufl.edu/) or at Beckman Coulter (http:/beckmangenomics.com/). Electropherograms were corrected by eye and aligned using Sequencher 4.8 (Gene Codes Corp.). Unique haplotypes were identified using DnaSP 5.10.1 (Rozas et al. 2003). Phylogenetic analysis of *ND-2* sequences was implemented in Garli (Zwickl 2006) on the Cipres cluster (Miller et al. 2010), using the HKY model (Hasegawa et al. 1985) as selected by jModeltest 2.1.4 (Guindon and Gascuel 2003; Darriba et al. 2012). An *ND-2* sequence of the triakid Tope *Galeorhinus galeus* was used as an out-group; support values for nodes were generated utilizing 1,000 bootstrap replicates. Phylogenetic trees were summarized using Sumtrees (Sukumaran and Holder 2010) and the consensus tree drawn using FigTree (Rambaut 2014). Pairwise genetic distances between the Smooth Dogfish, Florida Smoothhound, and Gulf Smoothhound were estimated as the proportion of variant sites (*p*-distance) (using mtDNA sequences) in Mega 6.06 (Tamura et al. 2013), and as Nei's genetic distance (Nei et al. 1983) (using microsatellite data) in MSanalyzer (Dieringer and Schlötterer 2003). Standard errors were estimated from 100 within-sample bootstrap replicates.

All 287 smoothhound sharks from the northern Gulf were assayed for allelic variation at 20 nuclear-encoded microsatellites. Descriptions of microsatellites, PCR primers, and reaction protocols are given in Giresi et al. (2011). Amplicons were electrophoresed on 6% polyacrylamide gels using an ABI 377 automated sequencer (Applied Biosystems) following the manufacturer's instructions. The resulting chromatograms were analyzed in Genescan 3.1.2 (Applied Biosystems), and alleles were scored by size in base pairs using Genotyper 2.5 (Applied Biosystems). Assignment of individuals, based on microsatellite genotypes, was implemented using the Bayesian clustering algorithm in Structure (Pritchard et al. 2000; Falush et al. 2007). Initially, genetic groups were defined using multilocus microsatellite genotypes of 10 individuals from each of three distinct clades identified by phylogenetic analysis of mtDNA sequences. To assess whether these individuals assigned to distinct groups and to determine whether there was a detectable level of admixture among the groups, the no-admixture model in Structure was employed with 10,000 permutations and a burn-in of 1,000 permutations for $K = 1$–5; runs for each value of K were replicated five times. Structure Harvester (Earl and von Holdt 2012) was employed to generate averaged likelihood scores for each value of K. The remaining 257 individuals were then assigned to groups by using the admixture model, setting K to the selected number of groups (three), and employing 10,000 permutations with a burn-in of 1,000 for each of five replicates.

Discriminate analysis of principle components (DAPC), using multilocus microsatellite genotypes, also was carried out using Adegenet (Jombart 2008) in R 3.0.2 (R Development Core Team 2013), with prior group membership defined by genetically identified species designation.

The 46 whole specimens were assigned to one of three distinct groups based on mitochondrial and microsatellite data. A variety of external morphological characters were compared among male and female specimens in each group to determine whether macroscopically visible, external characters that unambiguously distinguished among the groups could be identified. Additional individuals, including holotypes, of specimens of *Mustelus* housed at the Smithsonian National Museum of Natural History and the Biological Teaching and Research Collections at Texas A&M University–College Station were examined to assess whether morphological characters identified as unique to one of the three groups matched characters of type and other specimens. A list of the examined material may be found in Table S.2.

To test whether spatial and temporal factors might be indicators of species presence, a multifactorial analysis (MFA) was carried out using the FactomineR package for R (Lê et al. 2008). Because multiple individuals of a given species were often captured in the same sampling event and each sampling event had the same set of spatiotemporal data, the total data set was thinned to 147 unique observations in which only one individual of each species (if encountered) was entered for each sampling event. A two-dimensional plane of the MFA was then constructed using data on depth, month of capture, and longitude, with species identity overlain on the data points. We also tested whether the species-specific mean ($\overline{\mu}_i$) of each spatiotemporal factor was the same as the grand mean ($\overline{\overline{\mu}}$) for that factor across all sampling events (H_0: $|\overline{\mu}_i - \overline{\overline{\mu}} = 0$ for each species i) in an analysis of variance (ANOVA) framework by using the general linear hypothesis testing (GLHT) function available in the Multcomp package for R (Bretz et al. 2010). A simple, single-step methodology was employed for each factor to correct P-vales for multiple testing; the significance of $H_0 > 0$ was then assessed at $\alpha = 0.05$.

RESULTS

A total of 20 mtDNA haplotypes were recovered from 132 sampled individuals. Phylogenetic analysis of mtDNA sequences resolved four well-supported, reciprocally monophyletic clades (Figure 2). Three clades included smoothhound sharks caught in the Gulf, whereas the fourth included only smoothhound sharks caught in waters off French Guiana. One clade included the specimen of Smooth Dogfish caught off Cape Cod in the western Atlantic, where only Smooth Dogfish are known to occur; this clade was designated tentatively as Smooth Dogfish. A second clade from the Gulf included mature male specimens (determined by the presence of calcified claspers) that were smaller than 65 cm total length; this clade was designated tentatively as Florida Smoothhounds based on prior work by Heemstra (1973, 1997) that demonstrated a smaller size at maturity than for the other species. The third clade from the Gulf included several large specimens and was designated tentatively as Gulf Smoothhounds. Morphological assessment (below) confirmed these tentative species assignments. The fourth clade was assumed to represent Smalleye Smoothhounds, but no voucher material from French Guiana was available for examination. The distribution of mtDNA haplotypes among the four species of smoothhound sharks is given in Table 1; the mtDNA haplotype found in each of the 132 individuals assayed is given in Table S.3.

The results from the multilocus microsatellite assignment were consistent with the clades recovered by the phylogenetic analysis. Final assignment of individuals to the three clades was based on 15 microsatellites (Table S.4), as 5 microsatellites were either not diagnostic to an individual species or did not amplify across all species. The clade containing smoothhound sharks from French Guiana was not included in the Structure analysis because many microsatellites could not be amplified consistently from fin clips from these specimens. The most likely value of K was 3 ($P > 99\%$), and the assignment of individual smoothhound sharks was unambiguous: 132 were assigned to the Smooth Dogfish clade, 39 to the Florida Smoothhound clade, and 116 to the Gulf Smoothhound clade. Of the 287 individuals assayed, 84 ($\sim$29%) were either misidentified in the field (61) or identified only as an unknown species of *Mustelus* (23). The results of the DAPC analysis (Figure 3) corroborated the presence of three genetically distinct units and identified individuals that had been misclassified or not assigned to individual species. Pairwise genetic distances based on both mtDNA and microsatellites (Table 2) confirmed that all three species are genetically divergent from one another.

Comparisons of external morphology among the 46 whole specimens (divided into discrete groups and tentatively assigned to species based on analysis of mtDNA and microsatellites) revealed characters that can be used to distinguish among adult specimens (Supplementary Figures S.1–S.3). When laid flat, the Smooth Dogfish can be identified by the relatively straight posterior margins of its pelvic and pectoral fins and by nasal flaps that are medially expanded. Adult Florida Smoothhounds can be identified by an acutely pointed, posteriorly directed lower lobe of the caudal fin (as noted by Bigelow and Schroeder 1948 and Heemstra 1997). In addition, adult males can be identified by the presence of calcified claspers in individuals smaller than 65 cm total length (Heemstra 1997). Gulf Smoothhounds can be identified by very long, upper labial furrows that extend to a perpendicular line even with the symphysis of the lower jaw, by mostly biserial rows of ampullae of Lorenzini (the ventral group of outer buccal tubules [Chu and Wen 1979]) posterior to the upper labial furrows, and by nasal flaps that are narrow with an acute posterior margin. The ampullae in Smooth Dogfish and Florida Smoothhounds are posterior to the upper

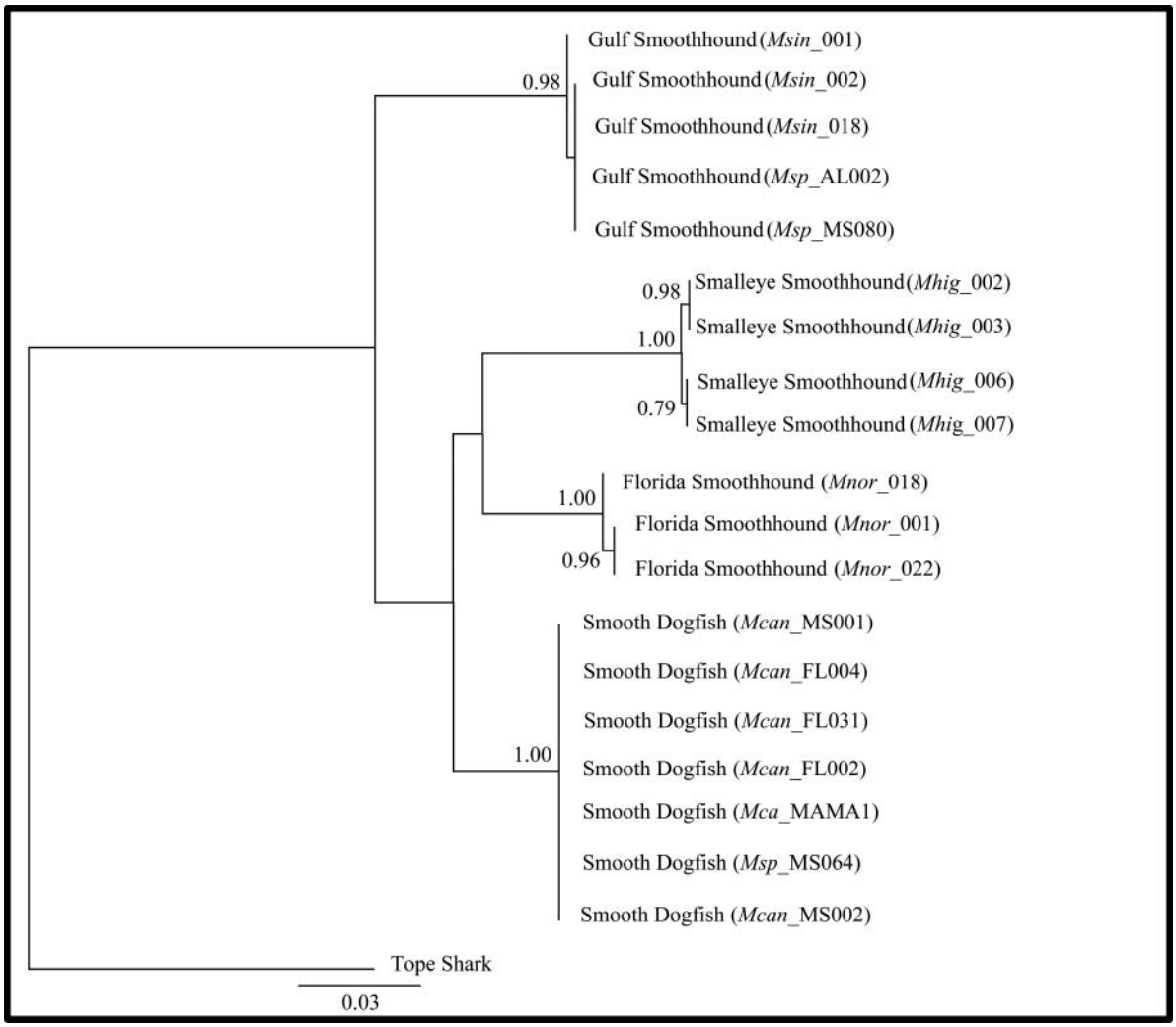


FIGURE 2. Phylogenetic relationships (gene tree) inferred from *ND-2* sequences of smoothhound sharks from the Gulf of Mexico and offshore of French Guiana. The numbers at the nodes are bootstrap support values; only values greater than 75% are shown. The Smooth Dogfish specimen labelled MAMA1 is the specimen captured near Cape Cod.

labial furrows and mostly uniserial, while the nasal flaps are medially expanded with relatively straight posterior margins. A dichotomous key can be found in Appendix 1.

The first two dimensions of the multifactorial analysis explained 75% of the variance and revealed that the distribution of individuals of the three species was not homogenous along the two axes (Figure 4); Florida Smoothhounds were found primarily in shallow waters, while Smooth Dogfish were found in the deepest waters. The estimated mean ± SE depth of capture for all three species (based on GLHT) followed the same pattern, differing significantly in pairwise comparisons from the estimated mean ± SE depth for all sampling events (138.13 ± 8.64 m): Florida Smoothhounds (15.80 ± 7.44 m, $t = -5.471$, $P < 0.001$), Gulf Smoothhounds (112.01 ± 6.51 m, $t = -2.64$, $P = 0.024$), and Smooth Dogfish (179.74 ± 13.36 m, $t = 5.86$, $P < 0.001$). Captures of Florida Smoothhounds were primarily in the eastern Gulf (also noted by Heemstra 1997), whereas captures of Smooth Dogfish and Gulf Smoothhounds occurred across the sampling area (Figure 1). The estimated mean month and longitude of capture of both Florida and Gulf Smoothhounds differed significantly from the estimated mean month (mid-July) and mean longitude (−88.60°) of all sampling events. The mean month and longitude of capture for Florida Smoothhounds were mid-May ($t = -3.20$, $P = 0.005$) and −85.60 ($t = 3.43$, $P = 0.002$), respectively, whereas the mean month and longitude of capture for Gulf Smoothhounds were early August ($t = 2.63$, $P = 0.024$) and −89. 63° ($t = -2.66$, $P = 0.022$), respectively. Both the estimated mean month and mean longitude of capture for Smooth Dogfish did not differ significantly from the estimated mean for all sampling events.

TABLE 1. Distribution of mtDNA haplotypes among four species of smoothhound shark.

mtDNA haplotype	Smooth Dogfish	Florida Smoothhound	Gulf Smoothhound	Smalleye Smoothhound	GenBank accession number
1				1	KP763703
2				3	KP763704
3			5		KP763705
4			15		KP763706
5			1		KP763707
6	41				KP763708
7	5				KP763709
8	5				KP763710
9	1				KP763711
10	8				KP763712
11		34			KP763713
12	3				KP763714
13		2			KP763715
14		1			KP763716
15				1	KP763717
16		1			KP763718
17		1			KP763719
18			1		KP763720
19			1		KP763721
20			2		KP763722

DISCUSSION

The genetic data (mtDNA sequences and microsatellite genotypes) obtained in this study are consistent with the occurrence of three genetically distinct species of smoothhound sharks in the northern Gulf of Mexico. Comparisons of external morphology among adult specimens from each clade with species descriptions and with type and other material from established collections permitted identification of each clade as one of the three species of *Mustelus* known from the northern Gulf. This allowed development of a morphological key that can be used to reduce misidentifications during routine field surveys, allowing for assessments of the abundance of each species. It is important to note that the key was tested rigorously only on adult specimens and that the key's utility in distinguishing among neonates and juveniles of the species is uncertain. The study also demonstrates the utility of combining molecular and morphological data to independently and unambiguously distinguish among difficult-to-identify species. Finally, the degree of genetic divergence in both mtDNA sequences and microsatellite genotypes in pairwise comparisons indicated that Florida Smoothhounds and Smooth Dogfish are genetically distinct and thus not the same species.

Multifactorial analysis and homogeneity tests of species-specific means versus grand means for depth, longitude, and month of capture for genetically identified smoothhound sharks revealed differences among the three species in preferred depth and between Florida Smoothhounds and Gulf Smoothhounds in average longitude and month of capture. Smooth Dogfish tend to prefer deeper waters (range, 64–408 m) than Gulf Smoothhounds (range, 51–233 m), while Florida Smoothhounds inhabit relatively shallow waters (1–92 m). Heemstra (1997) reported similar differences in depth of capture for Florida and Gulf Smoothhounds; however, the maximum depth found in this study for Smooth Dogfish (408 m) is greater than the depth (360 m) previously reported for the species (Heemstra 1997). The occurrence of Smooth Dogfish in deeper waters of the Gulf may be due in part to a preference for or tolerance of colder temperatures. This is consistent with the behavior of Smooth Dogfish along the East Coast of the United States, where the species migrates from the South Carolina coast northward to colder waters along the New England coast during the summer months and again heads southward during the winter months (Castro 2011; SEDAR 2014). Captures of Florida Smoothhounds were concentrated in the eastern Gulf, whereas captures of Gulf Smoothhounds tended to be farther to the west. There also was an apparent seasonal difference in time of capture between Florida Smoothhounds (late spring) and Gulf Smoothhounds (late summer). However, these regional and seasonal differences may have been biased by incomplete sampling across all regions in all seasons, and additional sampling is needed to further examine the patterns observed in this study.

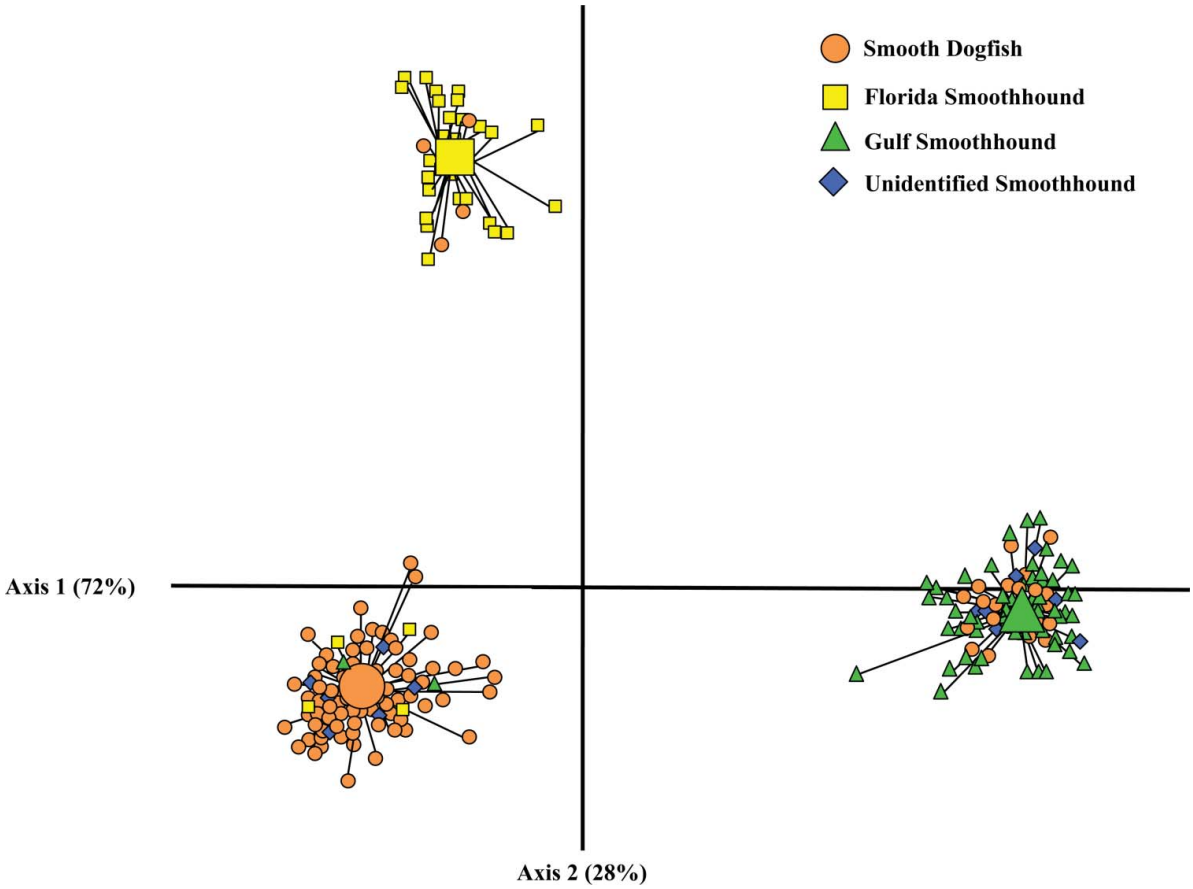


FIGURE 3. Discriminant analysis of principal components based on multilocus microsatellite genotypes of smoothhound sharks in the northern Gulf of Mexico. Cluster centroids are designated by the largest shapes. Individuals with different shapes than the centroid were either misidentified or not identified to species in the field. The proportion of variance explained by each axis is given. [Color figure available online.]

The sampling locations in this study were more or less consistent with those reported by Heemstra (1997), although we did find several Gulf Smoothhound individuals farther to the east than reported in Heemstra (1997). Captures of Florida Smoothhounds in both Heemstra (1997) and this study occurred primarily along the Florida Panhandle and on the West Florida Shelf, with only a few captures off the Alabama–Mississippi coast and the lower coast of Texas. However, because our sampling was limited during the winter months (December through February), we are unable to conclusively demonstrate differences in seasonal distribution. Consequently, more systematic sampling across time, depth, and geographic region is needed to fully decipher any temporal and spatial differences in distribution of all three species. No Smalleye Smoothhound individuals were recovered in the Gulf during the study. The lone Smalleye Smoothhound specimen reported from the northern Gulf was caught in DeSoto Canyon in 1970 at a depth of 1,281 m, 400 m deeper than reported for any other species of smoothhound and

TABLE 2. Mean ± SE pairwise genetic distances among three species of smoothhound sharks from the northern Gulf of Mexico. The values above the diagonal are *p*-distances derived from analysis of mtDNA, those below the diagonal are Nei's genetic distances derived from analysis of microsatellites. The SEs were estimated from 100 within-sample bootstrap replicates.

Species	Smooth Dogfish	Florida Smoothhound	Gulf Smoothhound
Smooth Dogfish		0.048 ± 0.006	0.051 ± 0.005
Florida Smoothhound	0.527 ± 0.009		0.072 ± 0.007
Gulf Smoothhound	0.556 ± 0.006	0.676 ± 0.005	

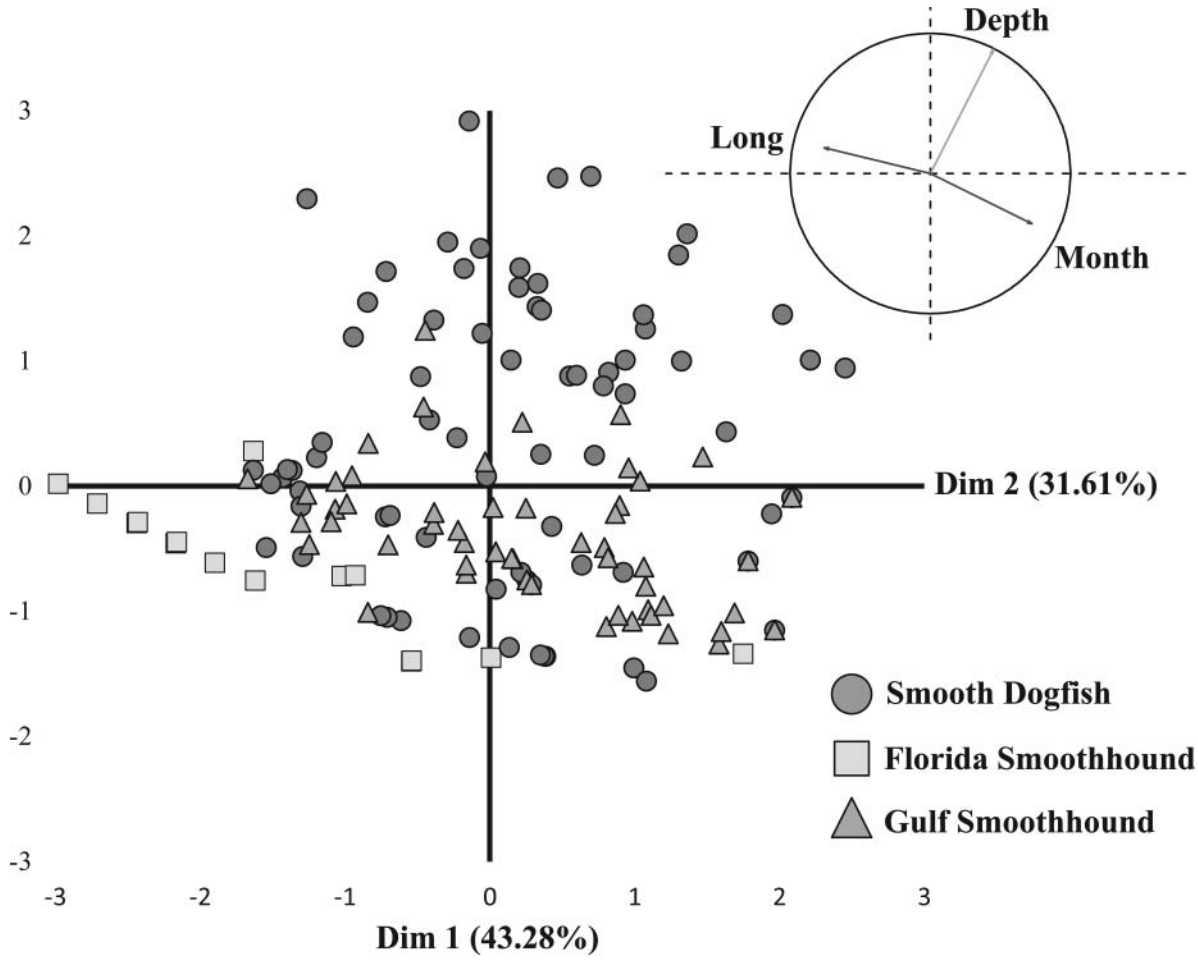


FIGURE 4. Multifactor analysis (MFA) of depth, month, and longitude for all sampling events for smoothhound sharks: circles = Smooth Dogfish, squares = Florida Smoothhound, and triangles = Gulf Smoothhound. The inset indicates the directionality of each factor on the MFA plane.

~800 m deeper than any other known records for the species (Heemstra 1973, 1997). Extensive longline sampling of DeSoto Canyon (320 stations between 200 and 2,000 m) occurred during this study, and only Smooth Dogfish were captured at depths greater than 400 m.

This study demonstrated the occurrence of three genetically distinct lineages of smoothhound sharks in the Gulf, identified as the Smooth Dogfish, Florida Smoothhound, and Gulf Smoothhound. Although the three species co-occur in the Gulf, they appear to have different depth preferences and perhaps spatiotemporal distributions. Our results also provide fisheries scientists with a simple morphological key with which to distinguish among these species in the field and indicate that the species may not be equally available to the fishery. To ensure that smoothhound shark management in the Gulf is based on the best available data, future studies to better understand life history differences among the three species and more systematic sampling across the Gulf are warranted.

ACKNOWLEDGMENTS

We thank G. Skomal (Massachusetts Division of Marine Fisheries); J. Imhoff and C. Peterson (Florida State University Coastal and Marine Laboratory); S. Gulak, K. Hannan, and C. Jones (National Oceanic and Atmospheric Administration); M. Drymon and A. Kroetz (Dauphin Island Sea Laboratory); T. Wiley-Lescher (Texas Parks and Wildlife Department); and M. Nalovic (Comité Regional de Peche à Maritime et Élevage Marine de Guyane) for assistance with the procurement of specimens and tissues. We also thank G. Naylor (University of Charleston) for providing an ND-2 sequence of *Galeorhinus galeus*; C. Caster, C. Hollenbeck, J. Puritz, and M. Renshaw for assistance in the laboratory; and B. Sterba-Boatwright for assistance with statistical analysis. This work was supported by the Cooperative Research Program of the National Marine Fisheries Service (NA12NMF4540083) and Texas AgriLife Research (Project H-6703). Field collections by R.D.G. were made possible by funding from the NOAA GulfSPAN Program and the Gulf of Mexico Research Initiative through the Florida Institute

of Oceanography and the Deep-C Consortium. This article is number 102 in the series Genetic Studies in Marine Fishes and publication number 8 of the Marine Genomics Laboratory at Texas A&M University–Corpus Christi.

REFERENCES

Bigelow, H. B., and W. C. Schroeder. 1948. Sharks. Pages 224–254 *in* J. Tee-Van, C. M. Breder, S. F. Hildebrand, A. E. Parr, and W. C. Schroeder, editors. Fishes of the western North Atlantic, part one. Lancelets, cyclostomes, sharks. Sears Foundation for Marine Research, Yale University, New Haven, Connecticut.

Bretz, F., T. Hothorn, and P. Westfall. 2010. Multiple comparisons using R. Chapman Hall/CRC Press, Boca Raton, Florida.

Campana, S. E., W. Joyce, L. Marks, P. Hurley, L. J. Natanson, N. E. Kohler, C. F. Jensen, J. J. Mello, H. L. Pratt, S. Myklevoll, and S. Harley. 2008. The rise and fall (again) of the Porbeagle shark population in the Northwest Atlantic. Pages 445–461 *in* D. Camhi, E. K. Pikitch, and E. A. Babcock, editors. Sharks of the open ocean: biology, fisheries and conservation. Blackwell Scientific Publications, Oxford, UK.

Castro, J. I. 2011. The sharks of North America. Oxford University Press, New York.

Chabot, C. L., and L. G. Allen. 2009. Global population structure of the Tope (*Galeorhinus galeus*) inferred by mitochondrial control region sequence data. Molecular Ecology 18:545–552.

Chu, Y. T., and M. C. Wen. 1979. Monograph of fishes of China: a study of the lateral-line canal system and that of Lorenzini ampulla and tubules of elasmobranchiate fishes of China. Science and Technology Press, Shanghai.

Compagno, L. J. V., and S. F. Cook. 1995. The exploitation and conservation of freshwater elasmobranchs: status of taxa and prospects for the future. Journal of Aquaculture and Aquatic Sciences 7:62–90.

Compagno, L., M. Dando, and S. Fowler. 2005. Sharks of the world. Princeton University Press, Princeton, New Jersey.

Cortés, E., and H. Balchowsky. 2014. Prelinimary catches of smoothhound sharks. Southeast Data, Assessment, and Review, SEDAR39-DW-0, North Charleston, South Carolina.

Darriba, D., G. L. Taboada, R. Doallo, and D. Posada. 2012. jModelTest 2: more models, new heuristics, and parallel computing. Nature Methods [online serial] 9:772.

Dieringer, D., and C. Schlötterer. 2003. Microsatellite analyzer (MSA): a platform-independent analysis tool for large microsatellite data sets. Molecular Ecology Notes 3:167–169.

Dulvy, N. K., J. D. Metcalfe, J. Glanville, M. G. Pawson, and J. D. Reynolds. 2000. Fishery stability, local extinctions, and shifts in community structure in skates. Conservation Biology 14:283–293.

Earl, D. A., and B. M. von Holdt. 2012. STRUCTURE HARVESTER: a website and program for visualizing STRUCTURE output and implementing the Evanno method. Conservation Genetics Resources 4:359–361.

Estoup, A., C. R. Largiader, E. Perrot, and D. Chourrout. 1996. Rapid one-tube DNA extraction for reliable PCR detection of fish polymorphic markers and transgenes. Molecular Marine Biology and Biotechnology 5:295–298.

Falush, D., M. Stephens, and J. K. Pritchard. 2007. Inference of population structure using multilocus genotype data: dominant markers and null alleles. Molecular Ecology Notes 7:574–578.

Giresi, M., M. A. Renshaw, D. P. Portnoy, and J. R. Gold. 2011. Isolation and characterization of microsatellite markers for the dusky smoothhound shark, Smooth Dogfish. Conservation Genetics Resources 4:101–104.

Guindon, S., and O. Gascuel. 2003. A simple, fast, and accurate algorithm to estimate large phylogenies by maximum likelihood. Systematic Biology 52:696–704.

Hasegawa, M., H. Kishino, and T. Yano. 1985. Dating of the human–ape splitting by a molecular clock of mitochondrial DNA. Journal of Molecular Evolution 22:160–174.

Heemstra, P. C. 1973. A revision of the shark genus *Mustelus* (Squaliformes: Carcharhinidae). Doctoral dissertation. University of Miami, Miami.

Heemstra, P. C. 1997. A review of the smoothhound sharks (genus *Mustelus*, family Triakidae) of the western Atlantic Ocean, with descriptions of two new species and a new subspecies. Bulletin of Marine Science 60:894–928.

IUCN (International Union for Conservation of Nature). 2013. Red list of threatened species. Available: www.iucnredlist.org. (February 2015.)

Jombart, T. 2008. adegenet: an R package for the multivariate analysis of genetic markers. Bioinformatics 24:1403–1405.

Lê, S., J. Josse, and F. Husson. 2008. FACTOMINER: an R package for multivariate analysis. Journal of Statistical Software 25:1–18.

Lopez, J. A., J. A. Ryburn, O. Fedrigo, and G. J. P. Naylor. 2006. Phylogeny of the sharks of the family Triakidae (Carcharhiniformes) and its implications for the evolution of carcharhiniform placental viviparity. Molecular Phylogenetics and Evolution 40:50–60.

Miller, M. A., W. Pfeiffer, and T. Schwartz. 2010. Creating the CIPRES science gateway for inference of large phylogenetic trees. Proceedings of the Gateway Computing Environments Workshop. Curran Associates, New Orleans, Louisiana.

Musick, J. A. 1999. Criteria to define extinction risk in marine fishes: the American Fisheries Society initiative. Fisheries 24(12):6–14.

Musick, J. A., G. Burgess, G. Cailliet, M. Camhi, and S. Fordham. 2000. Management of sharks and their relatives (Elasmobranchii). Fisheries 25(3):9–13.

Nei, M., F. Tajima, and Y. Tateno. 1983. Accuracy of estimated phylogenetic trees from molecular data. Journal of Molecular Evolution 19:153–170.

NMFS (National Marine Fisheries Service). 2010a. Guide for complying with the Atlantic shark fisheries regulations in Amendment 3 to the Consolidated Atlantic Highly Migratory Species Fishery Management Plan. NMFS, Silver Spring, Maryland.

NMFS (National Marine Fisheries Service). 2010b. Final Amendment 3 to the Consolidated Atlantic Highly Migratory Species Fishery Management Plan. NMFS, Silver Spring, Maryland.

Pritchard, J. K., M. Stephens, and P. Donnelly. 2000. Inference of population structure using multilocus genotype data. Genetics 155:945–959.

R Development Core Team. 2013. R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna. Available: http://www.R-project.org. (September 2015).

Rambaut, A. 2014. FIGTREE version 1.4.2. Available: http://tree.bio.ed.ac.uk/software/figtree/. (September 2015).

Rozas, J., J. C. Sanchez-Del Barrio, X. Messeguer, and R. Rozas. 2003. DNASP: DNA polymorphism analyses by the coalescent and other methods. Bioinformatics 19:2496–2497.

SEDAR (Southeast Data, Assessment, and Review). 2014. SEDAR 39 HMS Atlantic Smoothhound Shark Section: data workshop report. SEDAR, North Charleston, South Carolina.

SEDAR (Southeast Data, Assessment, and Review). 2015. SEDAR 39 HMS Gulf of Mexico Smoothhound Complex, section III: assessment process report. SEDAR, North Charleston, South Carolina.

Seutin, G., B. N. White, and P. T. Boag. 1991. Preservation of avian blood and tissue samples for DNA analyses. Canadian Journal of Zoology 69:82–90.

Springer, S., and R. H. Lowe. 1963. A new smooth dogshark, *Mustelus higmani*, from the equatorial Atlantic coast of South America. Copeia 1963:245–251.

Stevens, J. D., R. Bonfil, N. K. Dulvy, and P. A. Walker. 2000. The effects of fishing on sharks, rays, and chimaeras (chondrichthyans) and the implications for marine ecosystems. ICES Journal of Marine Science 57:476–494.

Sukumaran, J., and M. T. Holder. 2010. DENDROPY: a Python library for phylogenetic computing. Bioinformatics 26:1569–1571.

Tamura, K., G. Stecher, D. Peterson, A. Filipski, and S. Kumar. 2013. MEGA6: molecular evolutionary genetics analysis version 6. Molecular Biology and Evolution 30:2725–2729.

White, W. T., and P. R. Last. 2008. Description of two new species of gummy sharks, genus *Mustelus* (Carcharhiniformes: Triakidae), from Australian waters. Pages 189–202 *in* P. R. Last, W. T. White, and J. J. Pogonoski, editors. Descriptions of new Australian chondrichthyans. Australia Commonwealth Scientific and Industrial Research Organisation, Hobart.

Zwickl, D. J. 2006. Genetic algorithm approaches for the phylogenetic analysis of large biological sequence data sets under the maximum likelihood criterion. Doctoral dissertation. University of Texas, Austin.

Appendix 1: Dichotomous Field Key for Smoothhound Sharks in the Northern Gulf of Mexico

1a. Upper labial furrow noticeably longer than lower labial furrow, extending to a perpendicular line even with the symphysis of the lower jaw; ampullae of Lorenzini posterior to the upper labial furrow mostly biserial; nasal flaps narrow with a concave or angular posterior margin ..... Gulf Smoothhound

1b. Upper labial furrow only slightly longer than or the same size as lower labial furrow; ampullae of Lorenzini immediately posterior to upper labial furrow mostly uniserial; base of nasal flaps expanded medially with nearly straight posterior margin ..... go to 2

2a. Pectoral fin rear tip broadly rounded; posterior margin of pectoral and pelvic fins nearly straight; margin of lower lobe of caudal fin nearly straight with a rounded lobe; males mature greater than 80 cm total length ..... Smooth Dogfish

2b. Pectoral fin free, rear tips angular to narrowly rounded, posterior margins of pectoral and pelvic fins falcate; lower lobe of caudal fin pointed and directed posteriorly; males mature less than 65 cm total length ..... Florida Smoothhound

Transactions of the American Fisheries Society 144:1301–1310, 2015

DOI: 10.1080/00028487.2015.1069212

Supplement: Tables and Figures

Supplementary Table S.1. List of individual smoothhound sharks sampled in the northern Gulf of Mexico by time of sampling, location, and depth. Samples are arranged by correct species identification based on genetic data (mtDNA sequences and microsatellite genotypes). Sample numbers are those of individual surveys.

Sample no.	Year/month	Latitude	Longitude	Depth (m)
Genetically identified as Smooth Dogfish				
Mcan_MS016	2007/9	29.337	-87.774	107
Mcan_MS002	2008/11	28.855	-85.03	104
Mcan_MS013	2008/11	29.616	-86.157	77
Mcan_MS003	2009/1	28.653	-85.296	147
Mcan_MS023	2010/8	27.695	-95.649	279
Mcan_MS045	2011	28.196	-90.25	116
Mcan_MS006	2011/4			
Msp_MS055	2011/4	29.322	-87.848	99
Msp_MS056	2011/4	29.322	-87.848	99
Msp_MS057	2011/4	29.423	-87.861	68
Msp_MS082	2011/4	29.423	-87.861	68
Msp_MS086	2011/4	29.322	-87.848	99
Msin_004	2011/4	29.635	-86.925	236
Msp_MS097	2011/4	29.341	-87.857	99
Msp_MS099	2011/4	29.535	-86.734	68
Msp_MS121	2011/4	29.322	-87.848	99
Mcan_MS018	2011/5	29.308	-85.976	113
Msp_MS054	2011/5	28.893	-85.369	92
Mcan_MS005	2011/5	29.523	-87.393	109
Msp_MS068	2011/5	28.947	-85.542	92
Msp_MS107	2011/5	29.936	-86.465	64
Msp_MS102	2011/5	28.893	-85.369	92
Msp_MS103	2011/5	28.893	-85.369	92
Msp_MS116	2011/5	28.893	-85.369	92
Mcan_MS011	2011/6	29.523	-87.393	109
Msp_MS073	2011/6	27.351	-84.404	129
Msp_MS078	2011/6	27.351	-84.404	129
Msp_MS090	2011/6	27.668	-93.413	257
Msp_MS104	2011/6	29.423	-87.861	81
Msp_MS098	2011/6	27.851	-91.772	233
Msp_MS111	2011/6	27.351	-84.404	129

Sample no.	Year/month	Latitude	Longitude	Depth (m)
Msp_MS114	2011/6	28.579	-89.45	283
Msp_MS119	2011/6	27.351	-84.404	129
Mcan_MS004	2011/7	28.283	-85.48	
Msp_MS059	2011/7	27.941	-91.361	252
Msp_MS074	2011/7	26.875	-96.436	227
Msp_MS089	2011/7	28.055	-84.958	211
Msp_MS066	2011/7	26.66	-96.35	334
Msp_MS106	2011/7	29.379	-87.934	81
Msp_MS113	2011/7	29.079	-88.961	142
Msp_MS118	2011/7	29.857	-87.27	168
Mcan_FL002	2011/8	29.146	-86.279	297
Mcan_FL003	2011/8	29.073	-88.619	251
Mcan_FL004	2011/8	29.073	-88.619	251
Msp_MS081	2011/8	26.862	-96.4	310
Msp_MS070	2011/8	25.87	-84.319	185
Msp_MS112	2011/8	28.006	-84.623	99
Msp_MS091	2011/8	26.777	-84.552	408
Mcan_MS001	2011/9	27.237	-96.309	
Mcan_MS007	2011/9	27.559	-94.621	167
Mcan_MS009	2011/9	28.05	-90.723	24
Mcan_MS010	2011/9	28.817	-89.31	86
Mcan_MS012	2011/9	25.298	-84.345	276
Mcan_MS014	2011/9	28.034	-90.515	218
Mcan_MS017	2011/9	28.047	-90.663	161
Mcan_MS019	2011/9	26.313	-84.585	213
Msp_MS064	2011/9	28.204	-90.386	105
Msp_MS069	2011/9	27.507	-96.035	319
Mcan_MS024	2011/9	28.796	-85.116	81
Msp_MS105	2011/9	27.507	-96.035	185
Mcan_MS046	2011/10	29.806	-87.311	87
Mcan_MS051	2011/10	29.806	-87.311	87
Mcan_MS053	2011/10	29.806	-87.311	87
Mcan_MS026	2011/10	28.196	-90.25	116
Msp_MS076	2011/10	28.893	-85.369	196
Msp_MS084	2011/10	29.745	-87.232	206
Mcan_MS053	2011/10	29.806	-87.311	87
Msp_MS125	2011/10	29.706	-87.226	262
Mcan_FL044	2012/2			
Msp_AL005	2012/3	29.421	-88.724	257
Msp_AL006	2012/3	29.421	-88.724	197
Msp_AL007	2012/3	29.503	-87.593	68

Sample no.	Year/month	Latitude	Longitude	Depth (m)
Msp_AL008	2012/3	29.503	-87.593	68
Msp_AL009	2012/3	29.503	-87.593	75
Msp_AL010	2012/3	29.421	-88.724	75
Mcan_FL005	2012/4	26.806	-84.737	300
Mcan_FL006	2012/4	29.433	-87.295	404
Mcan_FL007	2012/4	29.07	-88.639	301
Mcan_FL008	2012/7	29.408	-87.359	408
Mcan_FL009	2012/7	29.301	-87.775	
Mcan_FL010	2012/7	29.307	-86.498	319
Mcan_FL011	2012/7	29.408	-87.359	408
Mcan_FL012	2012/7	29.519	-86.799	303
Mcan_FL013	2012/7	29.118	-86.134	251
Mcan_FL014	2012/7	29.144	-86.284	299
Mcan_FL015	2012/7	29.307	-86.498	319
Mcan_FL016	2012/7	29.297	-87.785	242
Mcan_FL017	2012/7	29.519	-86.799	303
Mcan_FL018	2012/7	29.474	-87.387	310
Mcan_FL019	2012/7	29.474	-87.387	310
Mcan_FL020	2012/7	29.118	-86.134	251
Mcan_FL021	2012/7	29.144	-86.284	299
Mcan_FL022	2012/7	29.408	-87.359	408
Mcan_FL023	2012/7	29.297	-87.785	242
Mcan_FL024	2012/7	29.307	-86.498	319
Mcan_FL025	2012/7	29.118	-86.134	251
Mcan_FL026	2012/7	29.307	-86.498	319
Mcan_FL027	2012/7	29.408	-87.359	408
Mcan_FL028	2012/7	29.474	-87.387	310
Mcan_FL029	2012/7	29.304	-86.337	258
Mcan_FL030	2012/7	29.519	-86.799	303
Mcan_FL031	2012/7	29.519	-86.799	303
Mcan_FL032	2012/7	29.408	-87.359	408
Mcan_FL033	2012/7	29.519	-86.799	303
Mcan_FL034	2012/7	29.144	-86.284	299
Mcan_FL035	2012/7	29.519	-86.799	303
Mcan_FL036	2012/10	29.303	-86.334	264
Mcan_FL037	2012/10	29.306	-86.492	330
Mcan_FL038	2012/10	29.3	-86.662	386
Mcan_FL039	2012/10	29.3	-86.662	386
Mcan_FL040	2012/10	29.148	-86.59	405
Mcan_FL041	2012/10	29.52	-86.8	319
Mcan_FL042	2012/10	29.52	-86.8	319

Sample no.	Year/month	Latitude	Longitude	Depth (m)
Mcan_FL043	2012/10	29.056	-88.595	300
Msp_MS130	2013	28.938	-88.77	313
Msp_MS131	2013			
Msp_MS132	2013	29.533	-87.437	76
Msp_MS133	2013	29.533	-87.437	76
Msp_MS134	2013	29.533	-87.437	76
Msp_MS135	2013			
Msp_MS142	2013/9	26.821	-96.451	203
Msp_MS143	2013/9	26.821	-96.451	203
Msp_MS144	2013/9	26.821	-96.451	203
Msp_MS154	2013/9	27.561	-96.045	142
Msp_MS170	2013/9	29.126	-88.751	82
Msp_MS171	2013/9	29.126	-88.751	82
Mcan_MS040	/5			
Mcan_MS020		28.05	-90.723	155
Mcan_MS030		29.806	-87.311	87
Mcan_MS015		29.62	-86.98	252
Genetically identified as Florida Smoothhounds				
Mnor_017	2002/4	30.024	-85.56	92
Mcan_MS022	2009/10	27.753	-95.772	74
Mnor_TX001	2010/5			
Mnor_TX002	2010/5			
Mnor_TX003	2010/5			
Mnor_001	2011	29.834	-84.485	1
Mnor_002	2011	29.834	-84.485	1
Mnor_004	2011/5	29.834	-84.486	1
Mcan_MS008	2011/5	29.409	-88.185	
Msp_MS126	2011/6	29.322	-87.848	27
Mcan_MS025	2011/11	29.458	-85.482	28
Mnor_003	2011/12	29.833	-84.492	1
Mnor_005	2012/3	29.831	-84.488	1
Mnor_006	2012/3	29.831	-84.488	1
Mnor_007	2012/3	29.831	-84.488	1
Mnor_008	2012/3	29.831	-84.488	1
Mnor_009	2012/3	29.831	-84.488	1
Mnor_010	2012/3	29.831	-84.488	1
Mnor_011	2012/3	29.831	-84.488	1
Mnor_012	2012/3	29.831	-84.488	1
Mnor_013	2012/3	29.831	-84.488	1
Mnor_014	2012/3	29.883	-84.501	2
Mnor_015	2012/3	29.883	-84.501	2
Mnor_016	2012/3	29.883	-84.501	2

Sample no.	Year/month	Latitude	Longitude	Depth (m)
Mnor_018	2013/1	29.834	-84.487	1
Mnor_020	2013/1	29.834	-84.487	1
Mnor_030	2013/2	29.833	-84.487	1
Mnor_021	2013/4	29.884	-84.501	2
Mnor_022	2013/4	29.835	-84.487	1
Mnor_023	2013/4	29.884	-84.501	2
Mnor_025	2013/4	29.835	-84.487	
Mnor_026	2013/4	29.835	-84.487	1
Mnor_027	2013/4	29.835	-84.487	1
Mnor_028	2013/4	29.835	-84.487	1
Mnor_029	2013/4	29.835	-84.487	1
Mnor_024	2013/5	29.834	-84.487	1
Mnor_019	2013/6	29.835	-84.486	3
Mnor_031	2013/6	29.835	-84.486	3
Msp_MS128				
Msp_MS129				
Genetically identified as Gulf Smoothhounds				
Msin_006	2011/4	28.047	-90.665	161
Msin_010	2011/4	28.047	-90.665	161
Msp_MS058	2011/4	28.22	-93.04	68
Msp_MS065	2011/4	28.22	-93.04	68
Msp_MS077	2011/4	28.22	-93.04	68
Msp_MS079	2011/4	28.22	-93.04	68
Msp_MS087	2011/4	28.22	-93.04	68
Msp_MS092	2011/4	28.22	-93.04	68
Msp_MS093	2011/4	28.553	-85.859	68
Msp_MS101	2011/4	28.22	-93.04	68
Msp_MS108	2011/4	28.22	-93.04	68
Msp_MS115	2011/4	28.22	-93.04	68
Msp_MS060	2011/5	29.936	-86.465	75
Msp_MS094	2011/5	29.936	-86.465	75
Msp_MS095	2011/5	29.936	-86.465	75
Msp_MS096	2011/5	29.936	-86.465	75
Msp_MS120	2011/5	29.936	-86.465	75
Msin_002	2011/7	28.627	-89.72	118
Msin_005	2011/7	28.097	-90.864	124
Msin_008	2011/7	28.64	-89.257	193
Msp_MS067	2011/7	25.117	-83.369	67
Msp_MS080	2011/7	29.101	-84.037	51
Msp_MS085	2011/7	26.124	-83.866	108
Msp_MS124	2011/7	27.95	-84.398	74
Mcan_MS033	2011/8			
Msp_AL001	2011/8	29.337	-88.052	93

Sample no.	Year/month	Latitude	Longitude	Depth (m)
Msp_AL002	2011/8	29.337	-88.052	93
Msp_AL003	2011/8	29.337	-88.052	93
Mcan_MS021	2011/9	25.896	-83.837	108
Msin_003	2011/9	28.097	-90.864	124
Msin_009	2011/9	28.047	-90.665	161
Msp_MS061	2011/9	29.341	-87.857	97
Msp_MS062	2011/9	29.341	-87.857	97
Msp_MS072	2011/9	29.341	-87.857	97
Msp_MS075	2011/9	29.374	-87.912	97
Msp_MS100	2011/9	29.341	-87.857	97
Msp_MS110	2011/9	29.341	-87.857	97
Msp_MS122	2011/9	29.341	-87.857	97
Msp_MS123	2011/9	29.341	-87.857	97
Msp_MS127	2011/9	29.341	-87.857	97
Msp_AL004	2011/9	29.422	-87.918	66
Mcan_MS027	2011/10	28.661	-89.482	124
Mcan_MS028	2011/10	28.301	-93.168	58
Mcan_MS029	2011/10	29.806	-87.311	87
Mcan_MS031	2011/10	28.132	-91.956	86
Mcan_MS032	2011/10	29.806	-87.311	87
Mcan_MS034	2011/10			
Mcan_MS035	2011/10	26.53	-96.455	99
Mcan_MS036	2011/10	28.078	-92.224	97
Mcan_MS037	2011/10	28.661	-89.482	124
Mcan_MS038	2011/10	29.806	-87.311	87
Mcan_MS039	2011/10	29.806	-87.311	87
Mcan_MS041	2011/10	28.661	-89.482	124
Mcan_MS042	2011/10	29.806	-87.311	87
Mcan_MS043	2011/10	29.806	-87.311	87
Mcan_MS044	2011/10	29.806	-87.311	87
Mcan_MS047	2011/10	29.806	-87.311	87
Mcan_MS048	2011/10			
Mcan_MS049	2011/10			
Mcan_MS050	2011/10	28.078	-92.224	97
Msin_001	2011/10	28.64	-89.257	193
Msin_007	2011/10	28.097	-90.864	124
Msp_MS071	2011/10	25.448	-83.843	117
Msp_MS083	2011/10	27.267	-84.259	108
Msp_AL011	2012/5	29.423	-88.005	75
Msp_AL012	2012/5	29.462	-87.706	99
Msp_AL013	2012/5	29.462	-87.706	233
Msp_AL014	2012/5	29.462	-87.706	68
Msp_AL015	2012/5	29.462	-87.706	129
Msin_018	2012/7	29.348	-87.783	102

Sample no.	Year/month	Latitude	Longitude	Depth (m)
Msin_019	2012/10	29.089	-88.63	202
Msin_020	2012/10	28.91	-88.961	162
Msp_Gulf001	2012/10			
Msp_Gulf002	2012/10			
Msp_MS136	2013/9	26.36	-96.478	68
Msp_MS137	2013/9	26.36	-96.478	68
Msp_MS138	2013/9	26.36	-96.478	68
Msp_MS139	2013/9	26.36	-96.478	68
Msp_MS140	2013/9	26.36	-96.478	68
Msp_MS141	2013/9	26.36	-96.478	68
Msp_MS145	2013/9	26.821	-96.451	203
Msp_MS146	2013/9	26.821	-96.451	203
Msp_MS147	2013/9	26.821	-96.451	203
Msp_MS148	2013/9	27.326	-96.473	97
Msp_MS149	2013/9	27.326	-96.473	97
Msp_MS150	2013/9	27.326	-96.473	97
Msp_MS151	2013/9	27.326	-96.473	97
Msp_MS152	2013/9	27.621	-96.338	79
Msp_MS153	2013/9	27.621	-96.338	79
Msp_MS155	2013/9	27.561	-96.045	142
Msp_MS156	2013/9	27.999	-94.552	67
Msp_MS157	2013/9	28.075	-93.442	82
Msp_MS158	2013/9	28.186	-93.097	69
Msp_MS159	2013/9	27.908	-92.681	218
Msp_MS160	2013/9	28.181	-92.519	72
Msp_MS161	2013/9	28.344	-92.298	60
Msp_MS163	2013/9	28.019	-92.96	101
Msp_MS164	2013/9	28.019	-92.96	101
Msp_MS165	2013/9	28.301	-89.98	123
Msp_MS166	2013/9	28.301	-89.98	123
Msp_MS167	2013/9	28.301	-89.98	123
Msp_MS168	2013/9	28.301	-89.98	123
Msp_MS169	2013/9	28.301	-89.98	123
Msp_MS172	2013/9	29.126	-88.751	82
Msp_MS173	2013/9	29.126	-88.751	82
Msp_MS174	2013/9	29.126	-88.751	82
Msp_MS175	2013/9	29.126	-88.751	82
Msp_MS176	2013/9	29.126	-88.751	82
Msp_MS177	2013/9	29.126	-88.751	82
Msp_MS178	2013/9	29.126	-88.751	82
Msp_MS179	2013/9	29.126	-88.751	82
Msp_MS180	2013/9	29.783	-86.414	82
Msp_MS181	2013/9	28.47	-85.281	170
Msp_MS182	2013/9	29.867	-87.195	99

Sample no.	Year/month	Latitude	Longitude	Depth (m)
Msp_MS183	2013/9	25.117	-83.369	83
Msp_MS184	2013/9	29.958	-86.56	75
Mcan_MS052	/10			

Supplementary Table S.2. Comparative material examined for external morphology of smoothhound sharks.

Smithsonian National Museum (USNM):

Smooth Dogfish: USNM 10429, USNM 25400 (2 specimens), USNM 164520, USNM 188078, USNM 33461, USNM 357675, USNM 76685, USNM 314706, USNM 49239, USNM 25348, USNM 221718, USNM 396897, USNM 86723 (head only), USNM 7301, USNM 28714, USNM 9324, USNM 195858

Florida Smoothhounds: USNM 106639 (holotype**)**, USNM 57369 (paratype, plus neonates), USNM 317610 (paratype), USNM 104333, USNM 400711, USNM 208075, USNM 201920 (paratype)

Gulf Smoothhounds: USNM 208345 (holotype), USNM 158585(paratype), USNM 179120 (3 specimens, paratypes) USNM116443 (paratype)

Smalleye Smoothhounds: USNM 156930 (Holotype), USNM 187697 (4 specimens, paratypes), USNM 221724 (paratype), USNM 187695, USNM 187721 (5 specimens, paratypes), USNM 187707

Biological Teaching and Research Collections (Texas A&M University–College Station):

Smooth Dogfish: Massachusetts: 15684.0; Gulf: 15686.01, 15687.01, 15589.01, 15726.01, 15725.01, 15723.01, 16384.01, 16385.01, 16386.01, 16387.01, 16388.01, 16389.01, 16390.01, 16391.01, 16392.01, 16393.01, 3114.01, 3165.01, 3285.01, 4211.01, 4211.06, 4437.01, 4519.01, 4520.01, 4521.01, 4522.01, 4523.01, 5140.02, 6329.19, 10769.01, 5261.01, 15589.01.

Florida Smoothhounds: 15681.01, 15682.01, 15683.01, 15685.01, 15686.01, 15727.01, 15728.01, 16394.01, 16395.01, 16396.01, 16397.01, 15688.01, 15724.01, 2176.01, 2603.1, 6522.01,

Gulf Smoothhounds: 15679.01, 4388.01, 4387.01, 2929.01, 2355.01, 2354.02, 2354.01

Supplementary Table S.3. MtDNA haplotype and GenBank accession number for each smoothhound shark specimen assayed for *ND-2* sequences.

Haplotype no.	GenBank no.	Specimen ID
Smooth Dogfish		
Haplotype06	KP763708	Mca_MAMA1
Haplotype06	KP763708	Mca_MS001
Haplotype06	KP763708	Mca_MS012
Haplotype06	KP763708	Mca_MS013
Haplotype06	KP763708	Mca_MS014
Haplotype06	KP763708	Mca_MS017
Haplotype06	KP763708	Mca_MS019
Haplotype06	KP763708	Mca_MS020
Haplotype06	KP763708	Mca_MS023
Haplotype06	KP763708	Mca_MS026
Haplotype06	KP763708	Mca_MS040
Haplotype06	KP763708	Mca_MS045
Haplotype06	KP763708	Mca_MS046
Haplotype06	KP763708	Mca_MS051
Haplotype06	KP763708	Mca_MS055
Haplotype06	KP763708	Mcan_FL005
Haplotype06	KP763708	Mcan_FL006
Haplotype06	KP763708	Mcan_FL007
Haplotype06	KP763708	Mcan_FL016
Haplotype06	KP763708	Mcan_FL017
Haplotype06	KP763708	Mcan_FL024
Haplotype06	KP763708	Mcan_FL035
Haplotype06	KP763708	Mcan_FL040
Haplotype06	KP763708	Msin_004
Haplotype06	KP763708	Msp_AL006
Haplotype06	KP763708	Msp_MS066
Haplotype06	KP763708	Msp_MS069
Haplotype06	KP763708	Msp_MS076
Haplotype06	KP763708	Msp_MS081
Haplotype06	KP763708	Msp_MS088
Haplotype06	KP763708	Msp_MS091
Haplotype06	KP763708	Msp_MS097
Haplotype06	KP763708	Msp_MS102
Haplotype06	KP763708	Msp_MS104
Haplotype06	KP763708	Msp_MS105
Haplotype06	KP763708	Msp_MS109
Haplotype06	KP763708	Msp_MS119
Haplotype06	KP763708	Msp_MS121
Haplotype06	KP763708	Msp_MS125
Haplotype06	KP763708	Msp_MS144
Haplotype06	KP763708	Msp_MS154
Haplotype07	KP763709	Mca_MS009
Haplotype07	KP763709	Mca_MS030

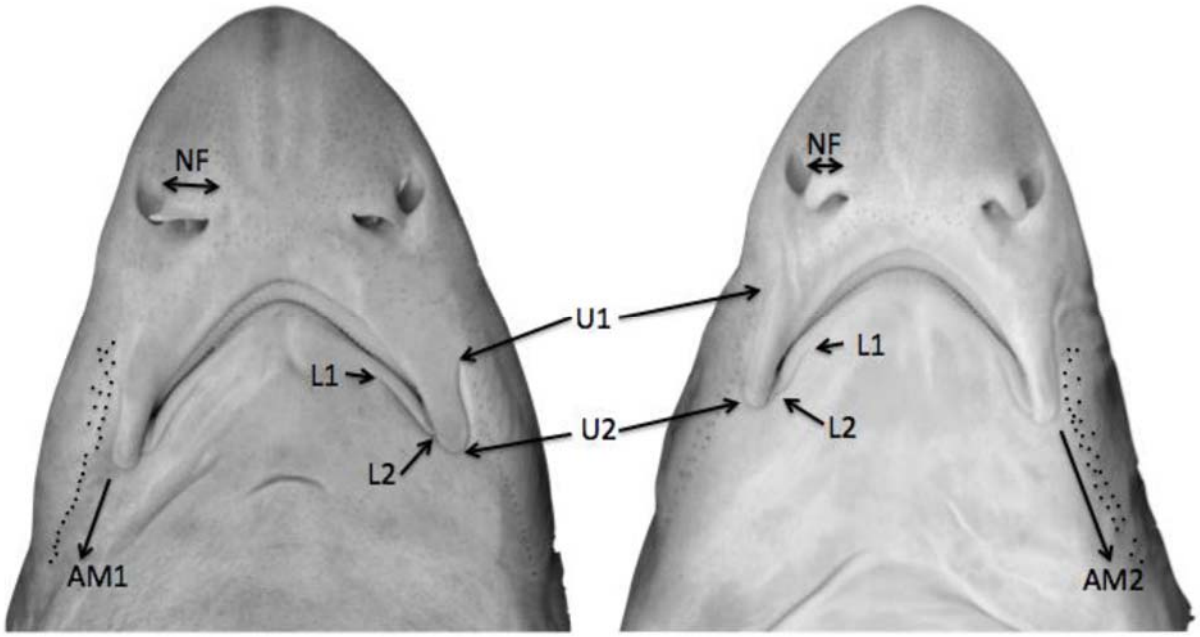
Haplotype07	KP763709	Mcan_FL002
Haplotype07	KP763709	Mcan_FL003
Haplotype07	KP763709	Msp_MS111
Haplotype08	KP763710	Mca_MS004
Haplotype08	KP763710	Mca_MS015
Haplotype08	KP763710	Mca_MS054
Haplotype08	KP763710	Mcan_FL031
Haplotype08	KP763710	Msp_MS116
Haplotype09	KP763711	Msp_MS064
Haplotype10	KP763712	Mca_FL004
Haplotype10	KP763712	Mca_FL011
Haplotype10	KP763712	Mca_MS053
Haplotype10	KP763712	Mca_MS053
Haplotype10	KP763712	Mca_MS099
Haplotype10	KP763712	Mcan_FL004
Haplotype10	KP763712	Mcan_FL011
Haplotype10	KP763712	Msp_MS099
Haplotype12	KP763714	Mca_MS002
Haplotype12	KP763714	Mcan_FL026
Haplotype12	KP763714	Msp_MS171
Florida Smoothhounds		
Haplotype11	KP763713	Mca_MS008
Haplotype11	KP763713	Mca_MS008
Haplotype11	KP763713	Mnor_001
Haplotype11	KP763713	Mnor_001
Haplotype11	KP763713	Mnor_002
Haplotype11	KP763713	Mnor_002
Haplotype11	KP763713	Mnor_003
Haplotype11	KP763713	Mnor_003
Haplotype11	KP763713	Mnor_006
Haplotype11	KP763713	Mnor_007
Haplotype11	KP763713	Mnor_008
Haplotype11	KP763713	Mnor_009
Haplotype11	KP763713	Mnor_010
Haplotype11	KP763713	Mnor_011
Haplotype11	KP763713	Mnor_012
Haplotype11	KP763713	Mnor_013
Haplotype11	KP763713	Mnor_014
Haplotype11	KP763713	Mnor_015
Haplotype11	KP763713	Mnor_016
Haplotype11	KP763713	Mnor_029
Haplotype11	KP763713	Mnor_029
Haplotype11	KP763713	Mnor_FL006
Haplotype11	KP763713	Mnor_FL007
Haplotype11	KP763713	Mnor_FL008
Haplotype11	KP763713	Mnor_FL009
Haplotype11	KP763713	Mnor_FL010
Haplotype11	KP763713	Mnor_FL011
Haplotype11	KP763713	Mnor_FL012

Haplotype11	KP763713	Mnor_FL013
Haplotype11	KP763713	Mnor_FL014
Haplotype11	KP763713	Mnor_FL015
Haplotype11	KP763713	Mnor_FL016
Haplotype11	KP763713	Mnor_MS126
Haplotype11	KP763713	Msp_MS126
Haplotype13	KP763715	Mca_MS022
Haplotype13	KP763715	Mnor_TX002
Haplotype14	KP763716	Mca_MS025
Haplotype16	KP763718	Mnor_018
Haplotype17	KP763719	Mnor_022
Gulf Smoothhounds		
Haplotype03	KP763705	Msin_001
Haplotype03	KP763705	Msin_009
Haplotype03	KP763705	Msp_MS085
Haplotype03	KP763705	Msp_MS123
Haplotype03	KP763705	Msp_MS153
Haplotype04	KP763706	Msin_002
Haplotype04	KP763706	Msin_003
Haplotype04	KP763706	Msin_006
Haplotype04	KP763706	Msin_011
Haplotype04	KP763706	Msp_AL001
Haplotype04	KP763706	Msp_AL003
Haplotype04	KP763706	Msp_AL004
Haplotype04	KP763706	Msp_AL004
Haplotype04	KP763706	Msp_Gulf002
Haplotype04	KP763706	Msp_MS061
Haplotype04	KP763706	Msp_MS071
Haplotype04	KP763706	Msp_MS072
Haplotype04	KP763706	Msp_MS173
Haplotype04	KP763706	Msp_MS177
Haplotype04	KP763706	Msp_MS178
Haplotype05	KP763707	Msp_MS080
Haplotype18	KP763720	Msin_018
Haplotype19	KP763721	Msp_MS139
Haplotype20	KP763722	Msp_AL002
Haplotype20	KP763722	Msp_Gulf001
Smalleye Smoothhounds		
Haplotype01	KP763703	Mhigmani_006
Haplotype02	KP763704	Mhigmani_001
Haplotype02	KP763704	Mhigmani_002
Haplotype02	KP763704	Mhigmani_008
Haplotype15	KP763717	Mhigmani_003

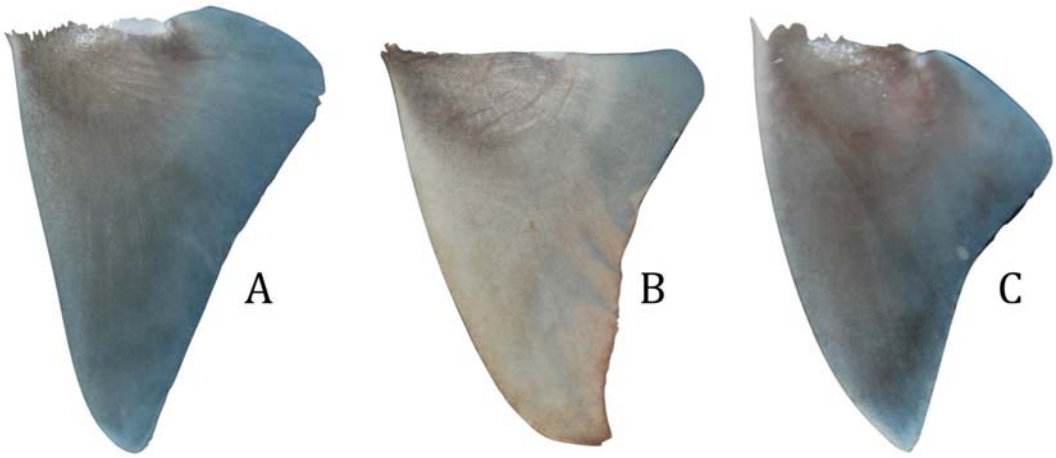
Supplementary Table S.4. Size range (bp) of alleles uncovered from amplifications of 15 microsatellites in three smoothhound shark species.

Microsatellite	Species	Range
Mca31	Smooth Dogfish	229-247
	Florida Smoothhounds	238
	Gulf Smoothhounds	226-238
Mca40	Smooth Dogfish	162-170
	Florida Smoothhounds	162
	Gulf Smoothhounds	160-164
Mca44	Smooth Dogfish	169-185
	Florida Smoothhounds	169-222
	Gulf Smoothhounds	159-222
McaB5	Smooth Dogfish	192-200
	Florida Smoothhounds	192-200
	Gulf Smoothhounds	196-218
McaB6	Smooth Dogfish	238-250
	Florida Smoothhounds	240-256
	Gulf Smoothhounds	238-250
McaB22	Smooth Dogfish	141-169
	Florida Smoothhounds	151-195
	Gulf Smoothhounds	135-171
McaB26	Smooth Dogfish	225-235
	Florida Smoothhounds	215-230
	Gulf Smoothhounds	220-230
McaB28	Smooth Dogfish	148-150
	Florida Smoothhounds	144-146
	Gulf Smoothhounds	130-150
McaB35	Smooth Dogfish	186-220
	Florida Smoothhounds	200-220
	Gulf Smoothhounds	202-214
McaB36	Smooth Dogfish	154-162
	Florida Smoothhounds	150-164
	Gulf Smoothhounds	152-162
McaB37	Smooth Dogfish	239-255
	Florida Smoothhounds	235-245

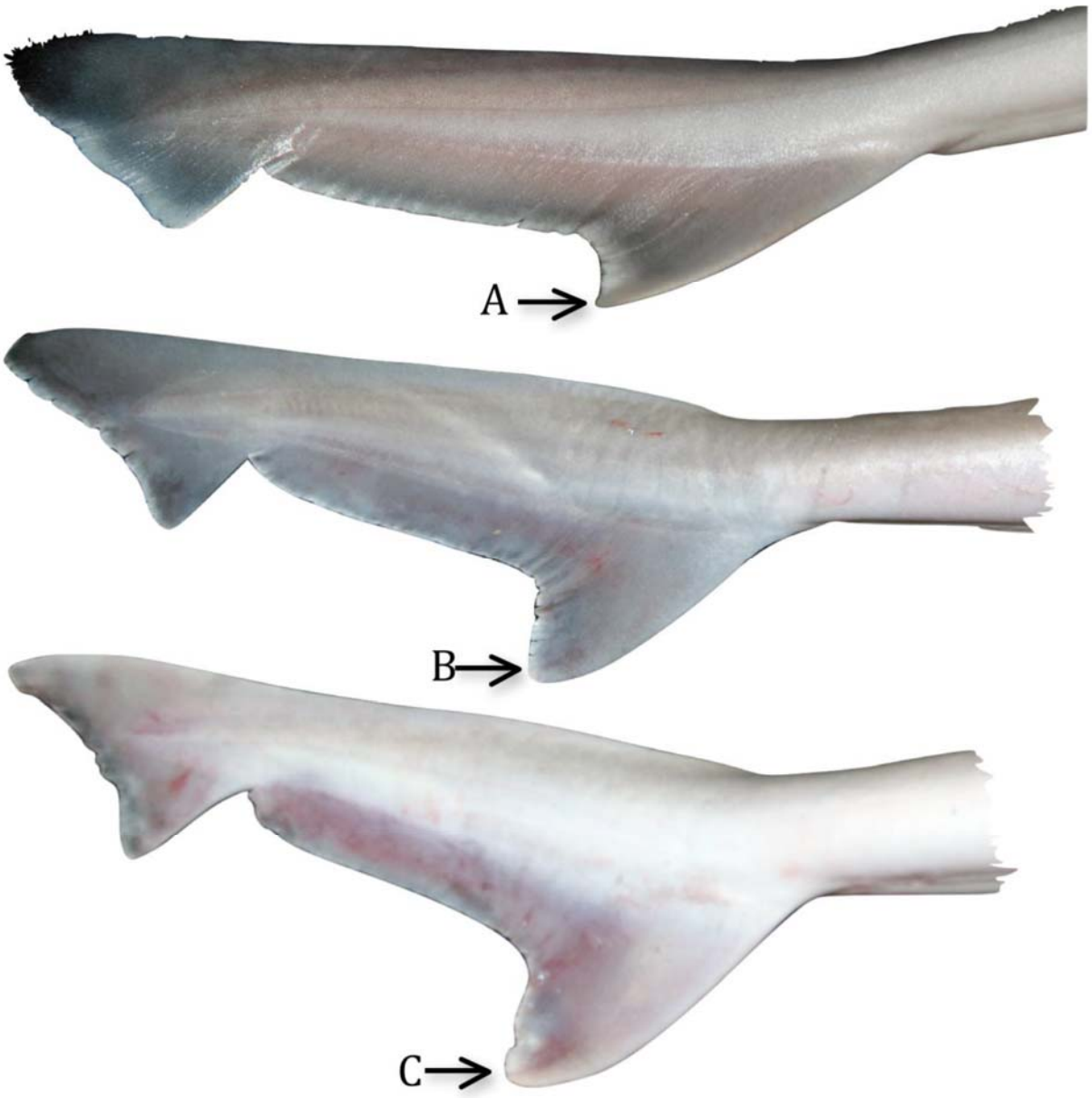
	Gulf Smoothhounds	241-253
McaB40	Smooth Dogfish	166-170
	Florida Smoothhounds	167-227
	Gulf Smoothhounds	170-215
McaB41	Smooth Dogfish	201
	Florida Smoothhounds	199
	Gulf Smoothhounds	199
Mca25	Smooth Dogfish	260
	Florida Smoothhounds	252-260
	Gulf Smoothhounds	252-262
MaWS1	Smooth Dogfish	181-193
	Florida Smoothhounds	187-195
	Gulf Smoothhounds	181-203



Supplementary Figure S.1. Differences on the ventral surface of the head among smoothhound shark species in the U.S. Gulf of Mexico. The specimen on the left is a Smooth Dogfish, the specimen on the right a Gulf Smoothhound. Abbreviations are as follows: NF = the anterior nasal flaps (medially expanded in the Smooth Dogfish); L1 = the anterior bound of the lower labial furrow, L2 = the posterior bound of the lower labial furrow; U1 = the anterior bound of the upper labial furrow, U2 = the posterior bound of the upper labial furrow; AM = the ampullae of Lorenzini directly posterior to the upper labial furrows, i.e., the ventral group of outer buccal tubules (AM1 shows one row of ampullae [Smooth Dogfish and Florida Smoothhound], while AM2 shows two rows of ampullae [Gulf Smoothhound]). The ampullae and the posterior margins of the nasal flaps have been darkened electronically for emphasis.



Supplementary Figure S.2. Pectoral fin comparison among smoothhound shark species in the northern Gulf of Mexico. The insertion into the body is located at the top left corner of each fin; the posterior margin is the rightmost edge. Panel **(A)** shows the pectoral fin of a Smooth Dogfish, which has a nearly straight posterior margin; panels **(B)** and **(C)** show the pectoral fins of a Gulf Smoothhound and a Florida Smoothhound, respectively, which have falcate posterior margins.



Supplementary Figure S.3. Comparison of the lower lobes of the caudal fins of **(A)** the Florida Smoothhound, in which the lobe is slightly falcate with an acute tip directed backwards, **(B)** the Smooth Dogfish, in which it is nearly straight with a rounded tip, and **(C)** the Gulf Smoothhound, in which it is falcate with a rounded tip that is angled backwards.