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

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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.

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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

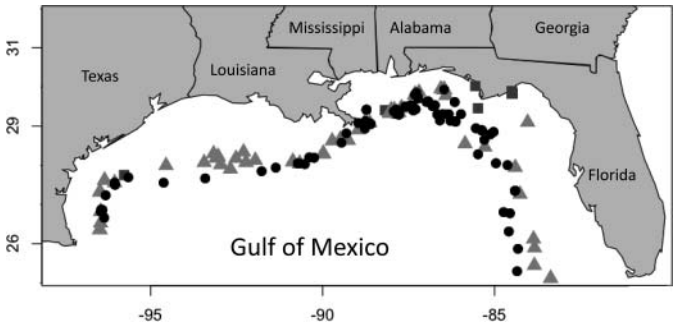

FIGURE 1. Locations from which smoothhound shark specimens were obtained in the northern Gulf of Mexico: circles = Smooth Dogfish, squares = Florida Smoothhound, and triangles = Gulf Smoothhound. A number of specimens were sampled from each locality (see Table S.1).

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

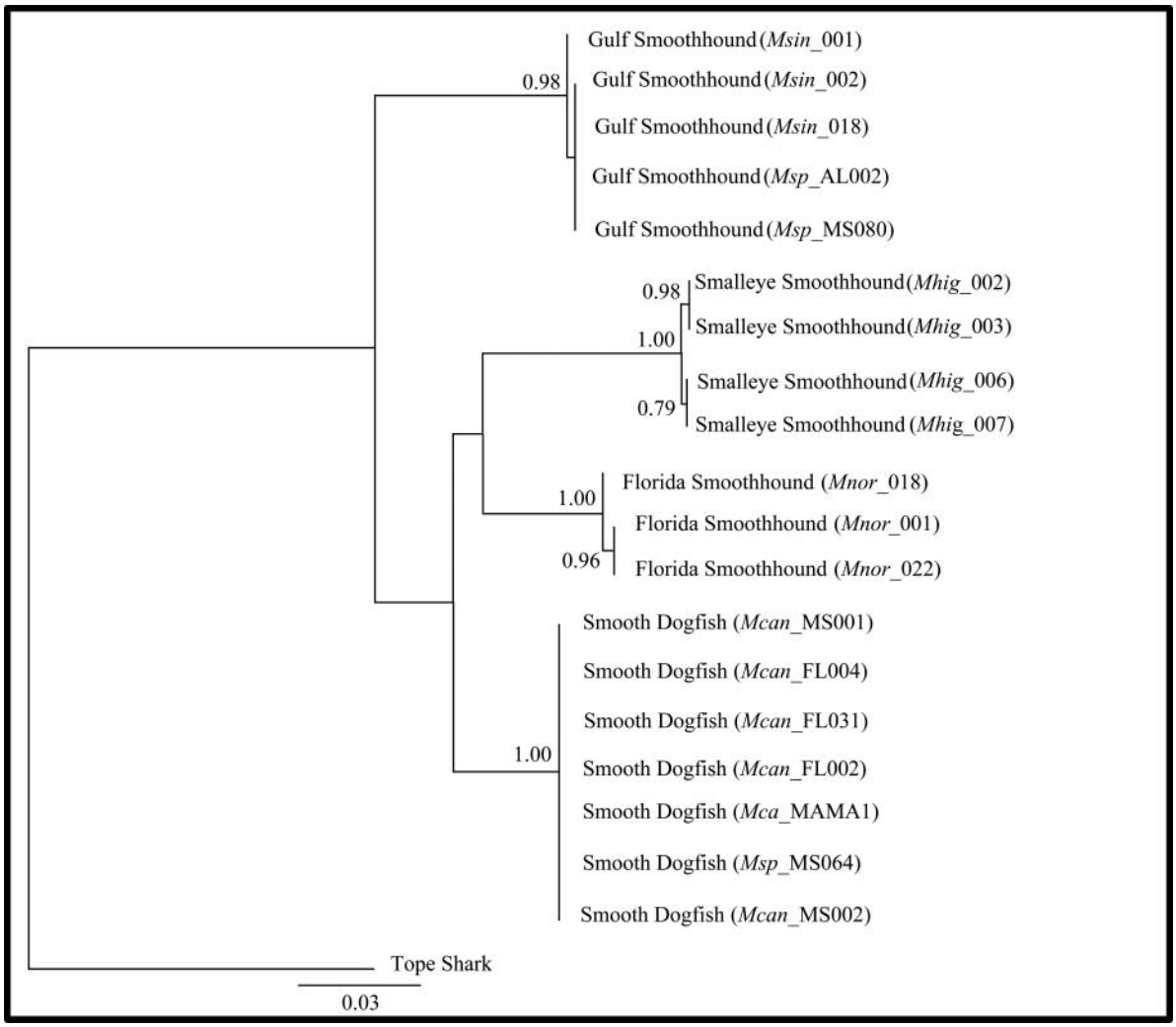

FIGURE 2. Phylogenetic relationships (gene tree) inferred from *ND-2* sequences of smoothhound sharks from the Gulf of Mexico and offshore of French Guiana. The numbers at the nodes are bootstrap support values; only values greater than 75% are shown. The Smooth Dogfish specimen labelled MAMA1 is the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

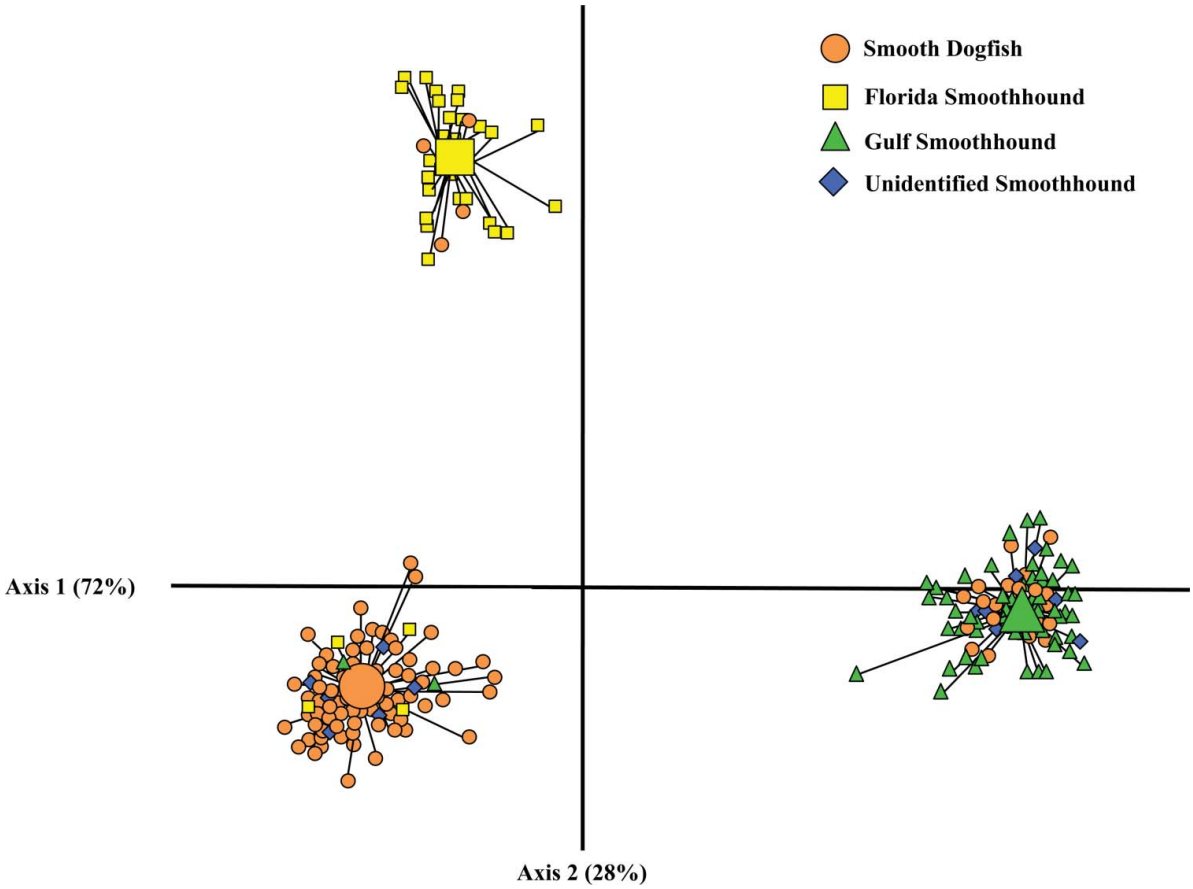

FIGURE 3. Discriminant analysis of principal components based on multilocus microsatellite genotypes of smoothhound sharks in the northern Gulf of Mexico. Cluster centroids are designated by the largest shapes. Individuals with different shapes than the centroid were either misidentified or not identified 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	

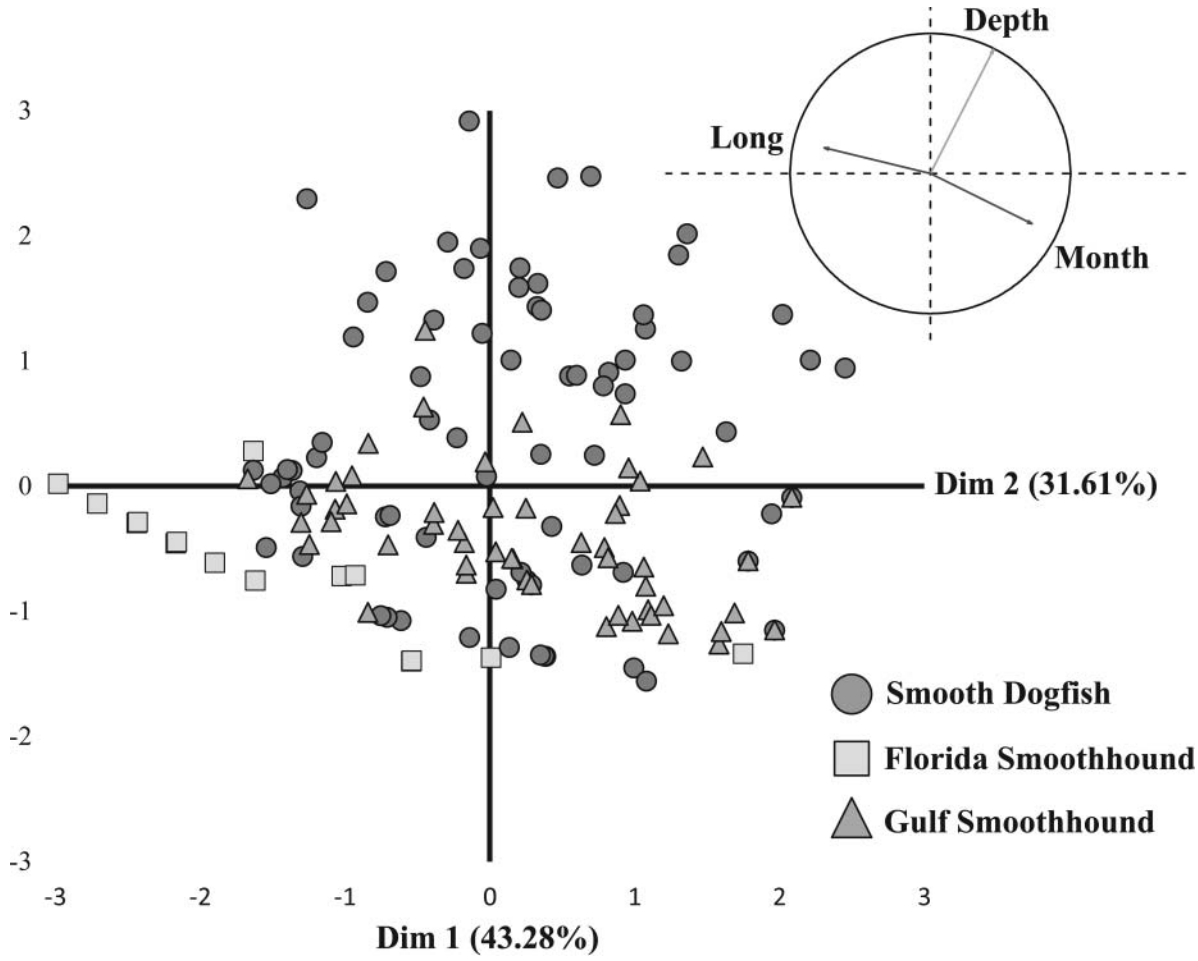

FIGURE 4. Multifactor analysis (MFA) of depth, month, and longitude for all sampling events for smoothhound sharks: circles = Smooth Dogfish, squares = Florida Smoothhound, and triangles = Gulf Smoothhound. The inset indicates the directionality of each factor on the MFA plane.

~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.

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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