POPULATION STRUCTURE OF THE DUSKY SMOOTHHOUND SHARK, *MUSTELUS CANIS,* IN U.S. WATERS AND IDENTIFICATION OF SPECIES IN THE GENUS *MUSTELUS* IN THE NORTHERN GULF OF MEXICO

A Dissertation

by

MELISSA GIRESI

Submitted to the Office of Graduate and Professional Studies of
Texas A&M University
in partial fulfillment of the requirements for the degree of

DOCTOR OF PHILOSOPHY

Chair of Committee,	Gil Rosenthal
Committee Members,	Charles Criscione
	Spencer Johnston
	Mary Wicksten
Head of Department,	Thomas McKnight

May 2016

Major Subject: Biology

ABSTRACT

Globally, the genus *Mustelus* (smoothhound sharks) represents one of the most speciose groups of cartilaginous fishes. Morphological similarities and geographic overlap among species cause difficulties with species identification and taxonomy. Four morphologically conserved species (*Mustelus canis canis*, *Mustelus sinusmexicanus*, *Mustelus norrisi* and *Mustelus higmani*) are thought to occur within the northern Gulf of Mexico (Gulf). Available morphological keys are inadequate to distinguish among these species, and as such, all smoothounds in the U.S. Atlantic will be be managed as a species complex.

The primary objectives of this study were to (i) develop and utilize molecular methods to distinguish among smoothhound species in the Gulf; (ii) identify morphological characters that can be used in field surveys to distinguish among the smoothhound species in the Gulf; (iii) test the null hypothesis that *Mustelus canis* is comprised of a single genetically panmictic stock in waters of the U.S. Atlantic (including the Gulf); (iv) assess genetic connectivity of *M. canis* in U.S. waters, and (v) to estimate the effective size and effective number of breeders from each locality sampled.

Phylogenetic analysis of sequences of the mitochondrially-encoded NADH-2 gene resolved three reciprocally monophyletic lineages, which were identified as *Mustelus canis, Mustelus norrisi,* and *Mustelus sinusmexicanus.* Concordant with these results, comparisons of multi-locus, nuclear-encoded microsatellite genotypes also resolved

three unambiguous groups. Using genetically verified voucher specimens, a field key outlining external characters was developed to aid field identification of the three species in the Gulf. Comparisons of environmental variables among specimens indicated that the three species, while co-distributed, might be partitioning the habitat based on depth and/or temperature tolerance.

Comparisons of ND-2 sequences and microsatellite genotypes among *M. canis* from localities throughout the U.S. Atlantic (including the northern Gulf of Mexico) rejected the null hypothesis that *M. canis* in U.S. waters of the western Atlantic comprises one genetically panmictic stock. Low but significant genetic structure was found between *M. canis* in the Gulf and the Atlantic, and also within ocean basins. The results of these studies have important implications for fisheries management of smoothhound sharks in the United States.

DEDICATION

I dedicate this work to my family for their unconditional support of my academic endeavors. My parents, Antonino Giresi Sr. and Patricia Giresi, and brothers, Patrick Sinclair, Matthew Sinclair, and Tony Giresi have always supported my academic and professional pursuits, including moves to Texas and New Zealand. My sister-in-law, Liz Sinclair, and brother-in-law, Marty Forth, always make sure that I'm included in the family debauchery, even from afar. Even though I don't get to see them nearly enough, I also dedicate this to my niece, Isabella Sinclair, and my nephews, Joseph Sinclair and Grayson Forth-Sinclair, who make me smile every time I get to see them via FaceTime or in person. I also dedicate this work to Les Kaufman, without whom, I would never have attended the Boston University Marine Program and or pursued this career.

ACKNOWLEDGEMENTS

First, I would like to thank my committee for their support over the last six and a half years. My chair, Gil Rosenthal has been a fantastic mentor over the last decade. The advice, support and guidance of my committee members, Charles Criscione and Spencer Johnston, have also been indispensable over the last six years. Dr. Mary Wicksten intimidated me when I came to recruiting weekend seven years ago, but she has been an inspiration to me, as a strong female scientist and as a mentor; I am grateful that she agreed to be added to my committee. Even when I've doubted myself, my committee has stood behind me, and for that, I am immensely grateful. Although John Gold was removed from the committee for logistical reasons, I am indebted to him for pushing me to pursue a Ph.D. instead of a Master's degree and for making this research possible. And though circumstances didn't allow for him to officially be a committee member, I am immensely grateful to David Portnoy, who has been my mentor, source of inspiration (and of laughter and jokes), and go-to person, for analyses, questions and guidance for the last seven years. Without Dave, this work would not have been possible; Dave has been a great honorary advisor.

I also owe thanks to Les Kaufman for encouraging me to pursue entry into the Boston University Marine Program, even though as a management student, I didn't fit any of the requirements. Thanks also to my Boston University Marine Program advisors, Gil Rosenthal, Gregory Skomal, and Paul Barber for being exemplary mentors and encouraging me to pursue a graduate degree. Thank you to all of following

individuals for sample collection, guidance and support over the last six years: Gregory Skomal and Jeremy King (Massachusetts Division of Marine Fisheries), Simon Gulak, William (Trey) Driggers, and Lisa Jones (NOAA), and Marcus Drymon and Andrea Kroetz (Dauphin Island Sea Lab), Cami McCandless (Apex Predators Program), Bryan Frazier (South Carolina DNR), Jim Gartland (VIMS), Dean Grubbs, Jo Imhoff, Cheston Peterson (Florida State University), Jim Gelschleiter, (UNF), and Tonya Wiley-Lescher (Texas Parks and Wildlife Department). I would like to thank my lab mates, present and former in both the Gold and Rosenthal labs, especially Mark Renshaw and Courtney Caster for assistance in the laboratory, and David Portnoy, Jon Puritz, Trevor Krabbenhoft, Eric Saillant, Ashley Hanna, and Ray Cui for assistance with data analyses. I thank Heather Prestridge and Kevin Conway of the Biological Research and Teaching Collections at Texas A&M University, and John McEachran (Professor Emertis at A&M) and Dean Grubbs (Florida State University) for assistance with morphological assessments.

I thank Jim Gelschleiter and lab (University of North Florida) for always making sure that I had a place to stay, regardless of what State/country we were visiting and Dean Grubbs (Florida State University) for being an exemplary collaborator and friend. John McEachran also deserves thanks for his advice, guidance and continued support over the last six years. The American Elasmobranch Society is full of amazing collaborators and colleagues and I am grateful to be a part of such a productive, social, and close-knit group of upstanding people and amazing scientists. I thank personnel at the Ichthyological Collections at both the Smithsonian National Museum and at the

American Museum of Natural History for their assistance. And, I thank my colleagues across the Ecology and Evolutionary Biology Interdisciplinary program at Texas A&M for encouragement and assistance.

For keeping me sane while finishing this work, I especially need to thank Emily Kasl, Emily Rose, Chris Nowotarski, Fred Zinnel, Ray Cui, Meaghan Pimsler, Justin Marinari, Jaime Rodriguez, Josephine Antwi, Juliana Rangel, Dana and Jason Park (Shark), Natalie Thompson, Pole and Jessica Klima, Theresa Southard, Lauren Vosper-Seals, Wing Siu, Joe Kudirka, and Jeff Guedry. Emily Kasl provided hardware, software, and cooked meals prior to my defense, and Chris Nowotarski was the only person to hear me practice my talk before the defense and provided invaluable advice, without these two people, I would not have finished this dissertation or made it through my defense. I am deeply grateful for their friendship and guidance. I thank my kickball and soccer teams for giving me an outlet for all my frustrations. And I thank the baristas (especially Zane) and regulars at Starbucks on University Drive for the caffeine, encouragement and friendship.

There was a point where I was going to leave science and give up on the degree together, but the friendships and guidance of Brian Bowen, Luiz Rocha, Steve Karl, Kevin Feldheim, Christi Wilcox, Kira Krend, Bryan Frazier, Tonya Darden, Costas Georghiades, Andrea Bonito, Courtney Schumacher, Sean Stroyick, Neal Rogers, Darren Henrichs and numerous others made me realize the potential in myself and refused to let me give up. Their friendships are invaluable. Again, I am immensely grateful for such great colleagues and collaborators.

Thank you to Biology Department for providing teaching assistantships over the last six years. The personnel in Introductory Biology and Anatomy made teaching infinitely easier and I am deeply appreciative to my colleagues in teaching programs for their assistance and encouragement. Thank you to Arne Lekven, Kay Goldman, Jennifer Bradford, and Will Bailey for helping to keep track of which department I was employed by and for ensuring that I was paid. I owe the final push to Jennifer Bradford and Arne Lekven for encouraging me to do what had to be done and finishing the degree. Dr. Lekven has gone above and beyond what is expected of a graduate advisor and for that, I deem him 'super-man'.

Work was supported by the Cooperative Research Program (CRP) of the National Marine Fisheries Service, U.S. Department of Commerce (NA12NMF4540083) and by Saltonstall-Kennedy Grant Program (NA10NMF4270218), which were awarded to Dr. John R. Gold and Dr. David S. Portnoy.

TABLE OF CONTENTS

Page

LIST OF FIGURES

LIST OF TABLES

CHAPTER I

INTRODUCTION TO THE STUDY SYSTEM

Species in the genus *Mustelus* are cartilaginous fishes belonging to the order Carcharhiniformes (ground sharks) and the family Triakidae (hound sharks). The family Triakidae is represented by 47 described species in nine genera (Eschmeyer 2012). The genus *Mustelus* (smoothhound sharks) contains 28 nominal species and a nominal subspecies (Compagno et al. 2005), which makes this genus one of the most specious genera of extent sharks.

Morphological overlap among species of *Mustelus* causes taxonomic confusion, makes it difficult to elucidate patterns of biogeography, and obscures the ability to obtain accurate fisheries statistics. Heemstra (1997) provided a taxonomic revision of the genus *Mustelus* in the western Atlantic Ocean based on morphological characters. However, the 'diagnostic' characters described (*Ibid*) to distinguish among species of *Mustelus* (position of fins, internarial distance, pattern of buccopharyngeal denticles and ridges on the dermal denticles, and labial furrow size) are highly variable, with considerable overlap among species (Heemstra 1997; Compagno 2005).

Confounding efforts to determine taxonomic relationships among species of *Mustelus* is geographic overlap among species (Boomer et al. 2010, Castro 2011). Members of the family Triakidae are found circumglobally; but only one member of the family Triakidae, the tope shark, *Galeorhinus galeus,* is cosmopolitan (Compagno et al. 2005). Many species have overlapping geographic ranges (IUCN 2015, Compagno

2005) and all described species of *Mustelus* are coastally distributed and found in temperature and subtropical waters.

Many species of *Mustelus* represent important fisheries resources according to the Internation Union for the Conservation of Nature's Red List (IUCN; www.iucnredlist.org) and some are susceptible to fisheries collapse. Of the 27 species of *Mustelus* for which status has been evaluated by the International Union for the Conservation of Nature, one is listed as Critically Endangered, one is listed as Endangered, two are listed as Vulnerable, two (including *M. canis)* are listed as Near-Threatened, eight are listed as Least-Concern, and the remainder (including *M. norrisi* and *M. sinusmexicanus)* are listed as Data-Deficient. Population trends have not been evaluated for most smoothhound species, but of those for which population trends have been evaluated, four (all in Central/South America) are experiencing population size declines, two have stable trends (Australia). Along the east coast of the United States, the dusky smooth hound shark, *Mustelus canis,* is one of the most commonly encountered sharks in coastal waters and is well-studied in terms of life history. However, studies of smoothhounds in the Gulf of Mexico, where the ranges of three species (the dusky smoothhound shark, *M. canis;* the Florida smoothhound shark, *Mustelus norrisi;* and the Gulf of Mexico smoothhound shark, *Mustelus sinusmexicanus)* are purported to overlap (Heesmtra 1997, Compagno 2005), are few.

Prior to the start of this study, there no stock or fisheries assessments of smoothhound sharks along the Atlantic coast of the U.S. had been carried out. The National Marine Fisheries Service (NMFS) recognized the need for an assessment of

stock structure of *M. canis* in U.S. waters and as such, the focus of the 39th South East Data Assessment and Review (SEDAR) was to determine stock status of smoothhound sharks in the U.S. Atlantic (Atlantic), and as part of the study, called for an assessment of genetic population structure among *M. canis* in the region. This study provided an assessment of genetic population structure among *M. canis* from localities throughout the east coast of the U.S. and northern Gulf of Mexico (Gulf).

The genetic assessments were useful in unambiguously distinguishing among the three species of smoothhounds in the Gulf and in identifying intra-specific genetic variability and divergence among *M. canis* in the Gulf and Atlantic. In the Final SEDAR report, it was recommended that smoothhounds in the U.S. Atlantic be managed as two stocks; one inclusive of all three species of *Mustelus* in the Gulf, and one inclusive of *M. canis* along the east coast of the U.S. The final reports also recommended that additional studies be carried out to assess the differences of life history and demography of the three species. Assessment of genetic and morphological differences among the species of *Mustelus*, as discussed in Chapter III of this dissertation, provided the tools by which the species can be identified and will benefit scientists and fishers who attempt to distinguish among the species in the field.

Biology of Smoothhounds in the U.S. Atlantic

Biology of Mustelus canis *in the Atlantic*

Along the east coast of the U.S, female *M. canis* reach maturity in four to five years at approximately 102 cm TL and live to a maximum of 16 years (Conrath et al. 2002); whereas males mature at approximately 85cm TL in two to three years and live to a maximum of 10 years (Conrath et al. 2002). The species is viviparous, with a yearly reproductive cycle that includes an 11-month gestation period (Conrath and Musick 2002). The largest female caught along the Atlantic coast was 130cm and the largest male was 112cm (Conrath et al. 2002). Females give birth to 3-18 well-developed pups (average 9.53) annually (Conrath and Musick 2002),

Mustelus canis uses shallow bays and estuaries as nurseries that presumably provide neonates with increased food resources and protection from predators (Skomal 2007, Conrath et al. 2002). There is some evidence that males and females may segregate by sex (Grubbs and Musick 2007; Skomal 2007). Skomal (2007) reported that 97% of individuals caught in long-line sets and 69% of individuals caught in gill nets off Cape Cod, Massachusetts, were female and that adult males were rarely caught in the same estuaries as females and neonates. Based on movement of gravid and post-partum females, and the presence of neonate and juvenile animals in estuarine and near-shore habitats (TeWinkle 1950; Conrath and Musick 2002; Skomal 2007), these habitats may be important nursery grounds.

Mustelus canis is abundant along the Atlantic coast of the U.S., but migrates

seasonally within this region. During the summer months, the range is contracted and this species is primarily found in the northern end of its range (New York, New Jersey, Cape Cod). Landings reported by Skomal (2007) indicate that the species is common in shallow coastal bays and estuaries in Cape Cod from mid-June through September. However, during the winter months, *M, canis* is most abundant in the southern part of the U.S. Atlantic (North Carolina, South Carolina, Georgia, Florida). During the spring and autumn, *M. canis* occupy the greatest geographic range in the U.S. Atlantic, which is likely due to the seasonal migrations to summer and wintering grounds (Bigelow and Schroeder 1948; Giresi et al 2015).

Biology of Mustelus canis *and* Mustelus sinusmexicanus *in the northern Gulf of Mexico*

Assessment of life-history parameters for *M. canis* in the Gulf were evaluated as part of the Southern East Data Assessment and Review (SEDAR) process (SEDAR 2014; Jones et al. 2014), but because of the inability to distinguish between *M. canis* and *M. sinusmexicanus,* the parameters were estimated as a function of both species combined. In the Gulf, females mature at a median age of 4.1 years at approximately 75.1 cm TL and they live to a maximum of 13 years (*SEDAR39-DW-22;* Jones et al. 2014). Males mature at a median age of 3.3 years at approximately 69.2 cm TL and live to a maximum of 11 years. The largest female caught in the Gulf was 129 cm and the largest male was 96.88 cm. Females have an annual reproductive cycle and give birth to 11-20 (average 15.5) well-developed pups annually.

Biology of Mustelus norrisi *in the northern Gulf of Mexico*

Mustelus norrisi is endemic to the Gulf of Mexico and no specimens of these species have been found along the Atlantic coast of the United States. Females of *M. norrisi* reach age to maturity at a median age of 4.1 years at approximately 58.5 cm TL (*SEDAR39-DW-22;* Jones et al. 2014). Males mature at a median age of 3.3 years at approximately 53.9 cm TL. Both males and females live to a maximum of 9 years. Females have an annual reproductive cycle and give birth to 8-14 (average 11.3) well-developed pups annually.

Project Objectives

The morphological similarity among smoothhound species causes taxonomic uncertainty and makes fisheries management decisions difficult (Heemstra 1997, Compagno 2005, Giresi et al. 2015). It is possible that one or more of the species in the Gulf may be more susceptible to fishing pressures than other species in the region. Genetic markers are useful for inferring geographic distributions, patterns of sexual/geographic isolation, and discovering distinct lineages. If distinct lineages are discovered, this can have important implications for conservation and fisheries management. The major objectives of this study were to first develop methods by which the species of smoothhounds in the Gulf could be distinguished from each other and second, to examine patterns of genetic divergence among the dusky smoothhound shark,

Mustelus canis, in the U.S. Atlantic (including the east coast of the United States from Massachusetts through Georgia and from throughout the Gulf). Highly polymorphic molecular markers were used to distinguish among the species in the Gulf and to assess population structure of *M. canis*. Macroscopically visible morphological characters were identified and used to distinguish among species. The results of these studies were used in fisheries management efforts, as part of the SEDAR assessment for smoothhound sharks.

Chapter II describes the development and optimization of microsatellite loci developed from a genomic library of *M. canis.* Chapter III presents molecular and morphological methods to distinguish among the smoothhound species in the Gulf. A morphological key, based on macroscopically visible characters, by which to distinguish among the species, is presented. Chapter IV presents the study of genetic population structure of the dusky smooth hound shark, *Mustelus canis* in U.S. waters, based on nuclear microsatellite loci and the mitochondrial NADH-2 (ND-2 gene).

CHAPTER II

ISOLATION AND CHARACTERIZATION OF MICROSATELLITE MARKERS FOR THE DUSKY SMOOTHHOUND SHARK, *MUSTELUS CANIS*[*]

Introduction

The dusky smoothhound shark, *Mustelus canis,* is a small demersal shark found in temperate waters along the continental shelf of the western Atlantic Ocean from Maine (USA) to southern Argentina (Compagno et al. 2005). The species is currently listed as 'Near-Threatened' by the IUCN red-list (Conrath 2005) and little is known about its population structure. Bigelow and Shroeder (1948) hypothesized that there are several distinct stocks of *M. canis* throughout its range, suggesting that an assessment of stock structure for the species will prove important for future conservation of dusky smoothhound resources. Polymorphic nuclear-encoded microsatellites have proven useful for detecting population structure in elasmobranchs on both large and small scales (Plank et al. 2010; Portnoy et al. 2010). Here, we describe development and characterization of 28 microsatellites (15 polymorphic) from an enriched genomic library of *M. canis,* as well as characterization in *M. canis* of four microsatellites developed for the triakid sharks *Galeorhinus galeus (Gg*3*, Gg*16*;* Chabot 2011*)* and *Mustelus antarcticus (Ma*FYP, *Ma*WS1*;* Boomer 2010), respectively.

---

*Reprinted with permission from "Isolation and characterization of microsatellite markers for the dusky smoothhound shark, *Mustelus canis*" Giresi, M., M. A. Renshaw, D. S. Portnoy, and J. R. Gold. 2011. *Conservation Genetics Resources* 4: 101-104 by Springer Science.
The final publication is available at http://link.springer.com/article/10.1007/s12686-011-9484-6

Materials and Methods

Generation of the enriched genomic library followed procedures outlined in Renshaw et al. (2010). Two separate hybridization reactions were performed; one with 50pmol of 3'-biotin modified $(CA)_{13}$ and the other with $(CAT)_8$ and $(GAT)_8$ oligonucleotides. Hybridization mixtures were heated to 95°C for 10 min and then kept at 58°C [$(CA)_{13}$ hybridization] and 47°C [$(CAT)_8$ and $(GAT)_8$ hybridization] for 1.25 h. Enriched genomic fragments were ligated into the pCR®2.1-TOPO® vector (Invitrogen) and transformed into *Escherichia coli* (One Shot® TOP10 Chemically Competent Cells, Invitrogen). Positive (white) clones were sent to University of Florida's Interdisciplinary Center for Biotechnology Research (http://www.biotech.ufl.edu/) for sequencing with M13 primers. Sequences were edited and vectors trimmed with Sequencher 4.1 (Gene Codes). Primer pairs were developed using Primer3plus (http://www.bioinformatics.nl/cgi-bin/primer3plus/primer3plus. cgi).

Initial PCR reactions followed Boutin-Ganache et al. (2001) and employed a forward primer with an attached 21-bp tail sequence (5'-GCCTCGTTTATCAGATGTGGA-3') labeled with either 6-Fam, Hex or Ned (Dye Set D, Applied Biosystems) and an unlabeled reverse primer; forward and reverse primers were purchased from Integrated DNA technologies (IDT). Primer pairs yielding clean amplifications were run on 24 individuals to identify polymorphic microsatellites. Nineteen of the microsatellites (15 from the *M. canis* library and four from two other triakids) were polymorphic. All 32 microsatellites were characterized on an additional

67 individuals of *M. canis*; for amplifications of all but one polymorphic microsatellite, the forward primer was directly labeled with either Hex or 6-Fam. The 21-bp-tail protocol of Boutin-Ganache et al. (2001) was used to characterize alleles at *Gg*16 and alleles at the 13 monomorphic microsatellites developed from the *M. canis* library. All individuals assayed were obtained in Delaware Bay, USA. Amplicons were electrophoresed on an ABI 377 automated sequencer with a 400HD [Rox] Size Standard (Applied Biosystems). Allele sizing and calling were performed using Genescan® version 3.1.2 and Genotyper® version 2.5 software (Applied Biosystems).

Genetic variability for each microsatellite marker was measured as number of alleles, gene diversity (expected heterozygosity), and observed heterozygosity, as calculated in GDA (Lewis and Zaykin 2001). A Fisher's exact test, as implemented in GDA (Lewis and Zaykin 2001), was used to test for significant departures from expectations of Hardy-Weinberg equilibrium at each microsatellite. Microchecker version 2.2.3 (Van Oosterhout et al. 2004) was utilized to check for the presence of null alleles, large-allele dropout, and/or stuttering at each microsatellite.

Results and Discussion

Summary data for 32 microsatellites, 28 developed from the genomic library of *M. canis* and for four developed in the two other triakid sharks (Chabot 2011; Boomer 2010) are presented in Table 2.1. The number of alleles detected ranged from two (*Mca*33, *Mca*40, *Mca*B28, McaB40*, Mca*B41*, Gg*3*, Ma*WS1) to 14 (*Mca*B22); expected

heterozygosity ranged from 0.011 (*Ma*WS1) to 0.859 (*Mca*B22), while observed heterozygosity ranged from 0.011 (*Ma*WS1) to 0.798 (*Mca*44). Genotypes at *Mca*B36 deviated significantly from Hardy Weinberg (HW) expectations following sequential Bonferroni correction (Rice 1989). The probability (*P*) that genotypes at *Mca*B22 did not fit HW expectation was close to the Bonferroni-corrected significance value of 0.003; the corrected *P* value, however, was 0.068, suggesting that genotypes at *Mca*B22 are not necessarily out of HW equilibrium. Evidence of one or more null alleles at *Mca*B22 was suggested by analysis with Microchecker. Single base-pair shifts in the dinucleotide microsatellite *Mca*B40 were detected in three individuals, but the alleles were easily scored. The microsatellites characterized here will prove useful for population genetic studies of *Mustelus canis* and potentially for other species in the family Triakidae.

Table 2.1 Summary data for 32 microsatellites characterized in the dusky smoothhound shark, *Mustelus canis.*

Microsat	Primer Sequence (5'-3')[a]	GenBank[b]	Repeat[c]	Clone Size[d]	T_A[e]	N/N_A[f]	Range[g]	H_E[h]	H_O[i]	P_{HW}[j]
POLYMORPHIC MICROSATELLITES										
*Mca*31	GGCAGATCAGTTGAGGAAGG AATGGGGAGACTTCTCTTTGC	JN083992	$(ATC)_4$	237	55	91/4	229-247	0.399	0.407	0.048
*Mca*33	CATTTGAACCCCGACAGAAC TCCAAGTAAGGATGAGTGACACC	JN083993	$(ATC)_5$	201	58	91/2	197-200	0.022	0.022	1.000
*Mca*40	AGCTCTGTCCAATCCAAGCT CAATTTATTATTGTTCAGAT	JN083994	$(AC)_5$	170	58	88/2	162-170	0.488	0.443	0.393
*Mca*44	TTTCCGCTGTATCACACATACAC GCATCTATATGTCTGCGTGTGTC	JN083995	$(AC)_{11}$	179	58	90/10	169-187	0.772	0.800	0.048
*Mca*B5	TAATCGACACGCAGTCATCG AAGCTCCAATTCTCACTGTGC	JN083996	$(GT)_{11}$	196	52	91/5	192-212	0.626	0.593	0.851
*Mca*B6	AGGATAAATACACGCACACAGG TTTTTGTTTTGCAATCTCACG	JN083997	$(CA)_{10}$	248	52°	91/7	238-254	0.186	0.165	0.017
*Mca*B22	TCCTCTCCAGGACAAACACAC TCCCACCTGCCATAGTAATTG	JN083999	$(AC)_{18}$	168	62	90/14	139-173	0.859	0.744	0.004
*Mca*B26	ACTGTGGCACTGCATTCTGC TGCATTTCAAAACCACTGGA	JN084000	$(AAATC)_5$	230	55	91/3	225-235	0.266	0.286	1.000
*Mca*B28	GGAGGAGCTAAGGGAAAAGC TCCTCAAGCTTCCAGAACACT	JN084001	$(TC)_8$	150	62	90/3	144-154	0.055	0.056	1.000

Table 2.1 Continued (2)

Microsat	Primer Sequence (5'-3')[a]	GenBank[b]	Repeat[c]	Clone Size[d]	T_A[e]	N/N_A[f]	Range[g]	H_E[h]	H_O[i]	P_{HW}[j]
*Mca*B33	TCTCCTAATGGAACGTGTGC GGTATGCGTATGGGTGTCG	JN084002	$(CA)_5$	155	55	91/5	154-166	0.522	0.593	0.566
*Mca*B35	AGTGCGTGCCAGTGTATGAG GTTCTGCATGGGACGTGAC	JN084003	$(TG)_8$	210	58	91/4	186-212	0.420	0.352	0.103
*Mca*B36	TTGGCTCGTTAAGGGTATGTG TTCTTTATCCCGTCGATTCC	JN084004	$(GT)_{10}$	155	62	91/3	150-164	0.531	0.451	0.002
*Mca*B37	TCTGCCTCTGTGTCTCATCC TTTCCATTTCCGACATAGGG	JN084005	$(GT)_5$	236	55	91/4	239-255	0.477	0.407	0.174
*Mca*B40	TGGCATTCCATTTGCTGATA TGTCAGCACAGGAGGGTGTA	JN084006	$(CA)_6$	170	64	90/5	166-171	0.507	0.511	0.199
*Mca*B41	TGTGCTATCACACGGAGTGG CTCACCCCCTCTCTTTCTCC	JN084007	$(TG)_5TT$ $T(GT)_2$ $(GA)_8$	207	58	90/2	205-209	0.427	0.389	0.443
*Gg*3	CCGTGACTGAAAGCAGCC CCCTCAACCATGGCAAGTG	N/A	$(GATT)_N$	*	58	91/2	241-249	0.022	0.022	1.000
Gg16	AGTGTGGTCTCACCAATGC TGGAAGGGTAAGGAAATTGGC	N/A	$(GA)_N$	*	N/A	90/4	184-190	0.518	0.544	0.851
*Ma*FYP	TGGTTGCCGATACAGCAGG CAAGCGCATGCACACTCAC	N/A	$(GT)_{11}(GT)_4$	*	58	91/8	238-260	0.760	0.725	0.473

Table 2.1 Continued (3)

*Ma*WS1	CGTAGCCAACCATTCCTGTT GAGCGTAGGGAGGTCAAGG	N/A	$(GT)_{15}$	*	60	91/2	181-191	0.011	0.011	1.000
Microsat	Primer Sequence (5'-3')[a]	GenBank[b]	Repeat[c]	Clone Size[d]	T_A[e]	N/N_A[f]	Range[g]	H_E[h]	H_O[i]	P_{HW}[j]
			MONOMORPHIC MICROSATELLITES							
Mca24	AAACTGCTGGCCTTGTCAAC AATCAGCACAAAGGGAGTGG	JN129144	$(GT)_5$	154	N/A	87/1	176	N/A	N/A	N/A
Mca25	ACACACTTTCACGCACAAGC TCGCTCAAGTGAGACCAGAG	JN129145	$(CA)_3(CT)_5$	240	N/A	85/1	260	N/A	N/A	N/A
Mca32	TCATTAAACCCGGACTTTGC CGACGAGCCTGATATGTGTG	JN129146	$(GA)_6$	237	N/A	90/1	258	N/A	N/A	N/A
Mca38	AATCAGCACAAAGGGAGTGG AAACTGCTGGCCTTGTCAAC	JN129147	$(AC)_5$	154	N/A	88/1	175	N/A	N/A	N/A
McaB4	TGTAAACAATCAGTGGCAAGC AAATTTGGAACGAGTGTCTGC	JN129148	$(CA)_7$	206	N/A	89/1	226	N/A	N/A	N/A
McaB7	CCTCGATGACTAATGCAAAGC GTGGGGACATGTTTGTGTGC	JN129149	$(CA)_5$	283	N/A	75/1	304	N/A	N/A	N/A
McaB16	AGGAGGATGCAGAGATTTGG ACTGATGCACGAGGACACC	JN129150	$(TG)_7$	196	N/A	88/1	208	N/A	N/A	N/A
McaB20	CCTTCAGGAAGGCAAAACC TTGGGTTTTAATGGGGATAGC	JN129151	$(AG)_6$	104	N/A	87/1	124	N/A	N/A	N/A
McaB21	CATGCCACGTGATAGTGAGG	JN129152	$(GA)_5$	169	N/A	85/1	190	N/A	N/A	N/A

Table 2.1 Continued (4)

Microsat	Primer Sequence (5'-3')[a]	GenBank[b]	Repeat[c]	Clone Size[d]	T_A[e]	N/N_A[f]	Range[g]	H_E[h]	H_O[i]	P_{HW}[j]
	TACCCCTCTGGTTCAAATGC									
McaB24	CGGGACACCGGAATAGATTA	JN129153	$(TG)_6$	243	N/A	77/1	255	N/A	N/A	N/A
	GATCAGATCCCTCCGTACCA									
McaB27	ATCCAGTGGTTTTGAAATGC	JN129154	$(GT)_6$	166	N/A	86/1	189	N/A	N/A	N/A
	CCTCGTAGGTCTCGTC									
McaB29	ACAATGGACACAGCAAGAGC	JN129155	$(AG)_7$	102	N/A	85/1	135	N/A	N/A	N/A
	CCCCTCTCAGTCTCACTCTCC									
McaB39	GGACAGGCAGCATCTGTGTA	JN129156	$(CA)_{10}GAT(AC)_8$	231	N/A	74/1	201	N/A	N/A	N/A
	CCCAGGGGGATTAGGATATT									

CHAPTER III

IDENTIFICATION AND DISTRIBUTION OF MORPHOLOGICALLY CONSERVED SMOOTHHOUND SHARKS (GENUS *MUSTELUS)* IN THE NORTHERN GULF OF MEXICO*

Synopsis

Identification of sharks within the triakid genus *Mustelus* (smoothhound sharks) is problematic because of extensive overlap among species in external morphology. Consequently, effective species-specific management of smoothhound resources is difficult when multiple species inhabit the same geographic region. Species identification and distribution of smoothhounds in the northern Gulf of Mexico (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 multi-locus 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 comparison with type and other specimens in established collections, the lineages were

*Reprinted with permission from "Identification and distribution of morphologically conserved smoothhound sharks (Genus *Mustelus)* in the northern Gulf of Mexico" Giresi, M.M., Grubbs, D., Portnoy, D.S., Drigger, W., Jones, L., and Gold, J.R. 2015. *Transactions of the American Fisheries Society* 144.6: 1301-1310. American Fisheries Society.

identified as *Mustelus canis, Mustelus norrisi*, and *Mustelus sinusmexicanus.* Two hundred and eighty-seven smoothhounds sampled from across the Gulf were then assigned unequivocally, based on genetic data, to each of the three species. Multi-factorial analysis and homogeneity tests of species-specific means versus grand means of spatial/temporal factors (depth, longitude, and month) at capture revealed significant differences among the three species in all three factors. *Mustelus canis* on average is found in deeper waters than *M. sinusmexicanus*, whereas *M. norrisi* inhabits relatively shallow waters. A diagnostic key for field identification of adult specimens of each species is provided.

Introduction

Global expansion of commercial and recreational shark fisheries over the last several decades has prompted concerns over 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; Campagna 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 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 cryptic, are highly susceptible to population collapse and/or local extirpation (Musick 1999; Dulvy et al. 2000).

Historically, several groups of sharks in U.S. waters have been managed as multi-species 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* contains 29 nominal species worldwide and is highly conserved in external morphology (Compagno et al. 2005; White and Last 2008). Globally, smoothhounds are important regional fisheries resources (Castro 2011; Compagno et al. 2005), and a number of species are listed as vulnerable, near-threatened, or endangered (IUCN 2013). The average, annual landings (commercial and recreational) of smoothhounds in U.S. waters of the western Atlantic Ocean (hereafter 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 smoothhounds in the Gulf (SEDAR 2015) is considered data poor or data limited because of the inability to discern among the three, possibly four nominal smoothhound species reported to occur in the Gulf (NMFS 2010a,b).

The four nominal species (Dusky Smoothhound, *Mustelus canis*; Florida Smoothhound, *Mustelus norrisi*; Gulf Smoothhound, *Mustelus sinusmexicanus*; and Small-eye Smoothhound, *Mustelus higmani*) are frequently misidentified due to the lack of clear and consistent external morphological characters that can be used reliably to distinguish among them (Heemstra 1997; Compagno et al. 2005). *Mustelus canis* is the most widely distributed of the four species, ranging from Massachusetts to northern

Brazil, and including the Gulf, and from southern Brazil through Argentina (Compagno et al. 2005). *Mustelus norrisi* has a more limited range and is reported to occur from the northern Gulf to Brazil (Heemstra 1997; Compagno et al. 2005); *M. sinusmexicanus* is thought to be endemic and restricted to the Gulf (Compagno et al. 2005). The fourth species, *Mustelus higmani*, was described originally (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 *M. higmani* was collected in the northeastern Gulf at a depth of >1,280 m, at least 400 m deeper than any prior recorded catches or sightings of a species of *Mustelus* (Heemstra 1997). Distributional data for *M. norrisi*, *M. sinusmexicanus*, and *M. higmani* are fairly limited and species designation of *M. norrisi* has been questioned (NMFS 2010a, b). Because reliable and consistent methods for distinguishing among these species of *Mustelus* in the field are unavailable, smoothhounds in U.S. waters of the Atlantic and Gulf are managed at present as a single, multi-species complex (NMFS 2010a, b).

Studies by Heemstra (1997) indicated that *M. norrisi* matures at smaller sizes than either *M. canis* or *M. sinusmexicanus*, and it is possible that other life-history characteristics (e.g., age at maturity, maximum age, fecundity) also may 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, unequivocal identification, stock status, and distribution of each smoothhound species in U.S. waters are needed for effective

conservation and management of smoothhound resources.

We assessed patterns of genetic divergence among smoothhounds sampled from U.S. waters of the Atlantic and Gulf, using sequences of mitochondrial (mt)DNA and nuclear-encoded microsatellites, to assess whether distinct genetic lineages (putative species) were present. We then executed detailed comparisons of external morphology on a subset of specimens from genetically distinct groups and identified each group to species by comparing specimens to 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 if there were predictive variables of species presence/absence across the Gulf.

Materials and Methods

A total of 287 adult smoothhound sharks were sampled from the Gulf (Figure 3.1) during bottom long-line, trawl, and/or gill-net surveys carried out between 2010 through 2013 by personnel from the Coastal and Marine Laboratory of Florida State University (FSUCML), the Mississippi Laboratories of the Southeast Fisheries Science Center, National Marine Fisheries Service/National Oceanographic and Atmospheric Administration (NMFS/NOAA), the Texas Parks and Wildlife Department (TPWD), and the Dauphin Island Sea Lab (DISL).

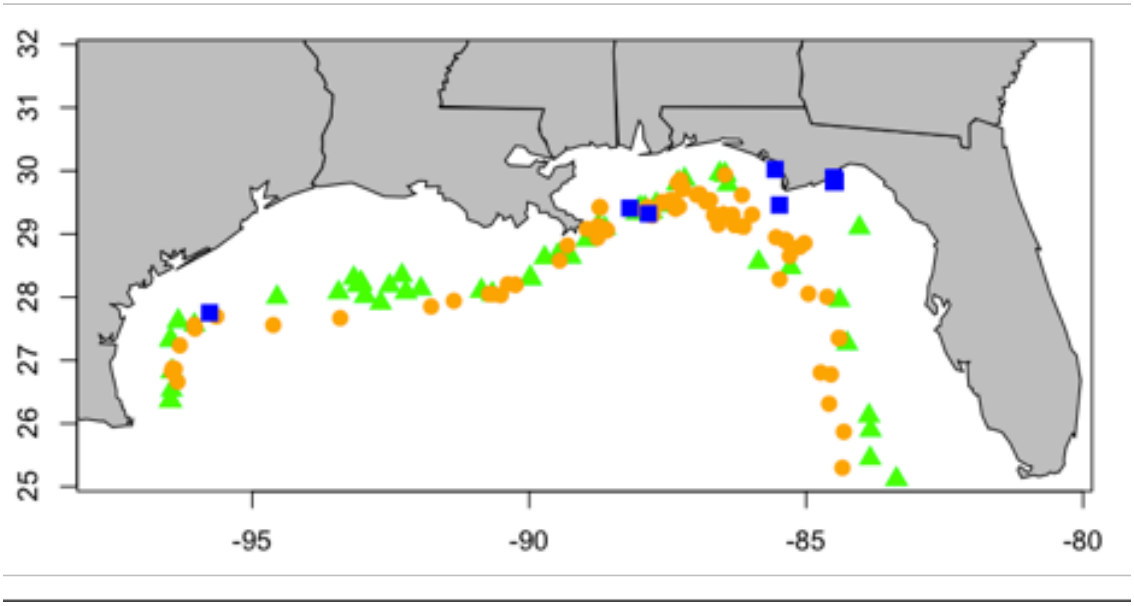

Figure 3.1 Locations of smoothhound specimens sampled in the northern Gulf of Mexico. *Mustelus canis* (circles), *M. norrisi* (squares), *M. sinusmexicanus* (triangles)

A single specimen of *M. canis*, 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 Table 3.1.

Table 3.1. List of individuals of *Mustelus* sampled from the northern Gulf of Mexico by year and season (month), location (latitude and longitude), and depth. Samples are arranged by sampling organization then by correct species identification based on genetic data (mtDNA sequences and microsatellite genotypes). Sample # is that of the sampling organization and the putative identification in the field.

Sample #	Year/Month	Latitude	Longitude	Depth
Genetically Identified as *Mustelus canis*				
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
Msp_MS114	2011/6	28.579	-89.45	283
Msp_MS119	2011/6	27.351	-84.404	129

Table 3.1 Continued (2)

Sample #	Year/Month	Latitude	Longitude	Depth
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			

Table 3.1 Continued (3)

Sample #	Year/Month	Latitude	Longitude	Depth
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
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

Table 3.1 Continued (4)

Sample #	Year/Month	Latitude	Longitude	Depth
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
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 *Mustelus norrisi*				
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

Table 3.1 Continued (5)

Sample #	Year/Month	Latitude	Longitude	Depth
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
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 *Mustelus sinusmexicanus*				
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

Table 3.1 Continued (6)

Sample #	Year/Month	Latitude	Longitude	Depth
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
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

Table 3.1 Continued (7)

Sample #	Year/Month	Latitude	Longitude	Depth
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
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

Table 3.1 Continued (8)

Sample #	Year/Month	Latitude	Longitude	Depth
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
Msp_MS183	2013/9	25.117	-83.369	83
Msp_MS184	2013/9	29.958	-86.56	75
Mcan_MS052	/10			

Fin clips (~1 cm^2) were taken either from the trailing edge of the first dorsal fin, the left pelvic fin, or the sub-terminal notch of the caudal fin and fixed in 20% DMSO storage

buffer (Seutin et al. 1991) or 95% ethanol. Tissue samples (fin clips) from 10 smoothhounds identified in the field as *M. higmani* 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 *M. canis* 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, 1x PCR buffer, 0.5 U *Taq* DNA polymerase (GoTaq Flexi DNA Polymerase, Promega), 1.5 uM 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 sec, 60°C for 1 min and 72°C for 1 min, and final extension of 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). PCR products were sequenced 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 *Galeorhinus galeus* (school shark) was used as an outgroup; 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 2009). Pairwise genetic distances between *M. canis*, *M. norrisi*, and *M. sinumexi*canus were estimated as the proportion of variant sites (*p*-distance), using mtDNA sequences, in Mega v6.06 (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 smoothhounds 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 manufacturer instructions. Resulting chromatograms were analyzed in Genescan® 3.1.2 (Applied Biosystems) and alleles were scored by size in base pairs (bp), 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 multi-locus microsatellite genotypes of ten 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 if 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 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. Discriminant analysis of principal components (DAPC), using multi-locus microsatellite genotypes, also was carried out using ADEGENET (Jombart 2008) in R v.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 (USNM) and the Biological Teaching and Research Collections (BTRC) 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 (Table 3.2).

Table 3.2 Comparative material examined for external morphology. The table includes the specimen ID, number of specimens in the lot, locality where the specimens are held, and indication of whether the specimens were type material.

SpecimenID	#Specimens	Location	Type
Mustelus canis			
USNM 10429	1	USNM	
USNM 25400	2	USNM	
USNM 164520	1	USNM	
USNM 188078	1	USNM	
USNM 33461	1	USNM	
USNM 357675	1	USNM	
USNM 76685	1	USNM	
USNM 314706	1	USNM	
USNM 49239	1	USNM	
USNM 25348	1	USNM	
USNM 221718	1	USNM	
USNM 396897	1	USNM	
USNM 86723	1	USNM	
USNM 7301	1	USNM	
USNM 28714	1	USNM	
USNM 9324	1	USNM	
USNM 195858	1	USNM	
15684	1	BRTC	
15686.01	1	BRTC	
15687.01	1	BRTC	
15589.01	1	BRTC	
15726.01	1	BRTC	
15725.01	1	BRTC	
15723.01	1	BRTC	
16384.01	1	BRTC	
16385.01	1	BRTC	
16386.01	1	BRTC	
16387.01	1	BRTC	
16388.01	1	BRTC	
16389.01	1	BRTC	
16390.01	1	BRTC	
16391.01	1	BRTC	
16392.01	1	BRTC	
16393.01	1	BRTC	
3114.01	1	BRTC	
3165.01	1	BRTC	
3285.01	1	BRTC	
4211.01	1	BRTC	
4211.06	6	BRTC	

Table 3.2 Continued (2)

SpecimenID	#Specimens	Location	Type
4437.01	1	BRTC	
4519.01	1	BRTC	
4520.01	1	BRTC	
4521.01	1	BRTC	
4522.01	1	BRTC	
4523.01	1	BRTC	
5140.02	2	BRTC	
6329.19	19	BRTC	
10769.01	1	BRTC	
5261.01	1	BRTC	
15589.01	1	BRTC	
15686.01	1	BRTC	
15687.01	1	BRTC	
15589.01	1	BRTC	
15726.01	1	BRTC	
15725.01	1	BRTC	
15723.01	1	BRTC	
16384.01	1	BRTC	
16385.01	1	BRTC	
16386.01	1	BRTC	
16387.01	1	BRTC	
16388.01	1	BRTC	
16389.01	1	BRTC	
16390.01	1	BRTC	
16391.01	1	BRTC	
16392.01	1	BRTC	
16393.01	1	BRTC	
3114.01	1	BRTC	
3165.01	1	BRTC	
3285.01	1	BRTC	
4211.01	1	BRTC	
4211.06	6	BRTC	
4437.01	1	BRTC	
4519.01	1	BRTC	
Mustelus norrisi			
USNM 106639	1	USNMH	Holotype
USNM 57369	1	USNMP	Paratype
USNM 317610	check	USNMP	Paratype
USNM 201920	1	USNMP	Paratype
USNM 104333	1	USNM	
USNM 400711	1	USNM	
USNM 208075	1	USNM	
15681.01	1	BRTC	

Table 3.2 Continued (3)

SpecimenID	#Specimens	Location	Type
15682.01	1	BRTC	
15683.01	1	BRTC	
15685.01	1	BRTC	
15686.01	1	BRTC	
15727.01	1	BRTC	
15728.01	1	BRTC	
16394.01	1	BRTC	
16395.01	1	BRTC	
16396.01	1	BRTC	
16397.01	1	BRTC	
15688.01	1	BRTC	
15724.01	1	BRTC	
2176.01	1	BRTC	
2603.1	1	BRTC	
6522.01	1	BRTC	
Mustelus sinusmexicanus			
USNM 208345	1	USNM	Holotype
USNM 158585	1	USNM	Paratype
USNM 179120	3	USNM	Paratype
USNM116443	1	USNM	Paratype
15679.01	1	BRTC	
4388.01	1	BRTC	
4387.01	1	BRTC	
2929.01	1	BRTC	
2355.01	1	BRTC	
2354.02	1	BRTC	
2354.01	1	BRTC	
Mustelus higmani			
USNM 156930	1	USNM	Holotype
USNM 187697	4	USNM	Paratype
USNM 221724	1	USNM	Paratype
USNM 187721	5	USNM	Paratype
USNM 187695	1	USNM	
USNM 187707	1	USNM	

In order to test whether spatial and/or 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 often

were captured in the same sampling event and each sampling event had the same set of spatial/temporal data, the total data set was thinned to 147 unique observations where 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 data points. We also tested whether the species-specific mean of each spatial/temporal factor (depth, longitude, and month) was the same as the grand mean for that factor across all sampling events (H_o: $_i$ − = 0; for each species, *i*) in an 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; significance of $H_o > 0$ 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 3.2). Three clades included smoothhounds caught in the Gulf, whereas the fourth included only smoothhounds caught in waters off French Guiana. One clade included the specimen of *M. canis* caught off Cape Cod in the western Atlantic where only *M. canis* is known to occur; this clade was designated tentatively as *M. canis*. 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 *M. norrisi*, based on prior work by Heemstra (1973, 1977) that demonstrated a smaller size at maturity for *M. norrisi*

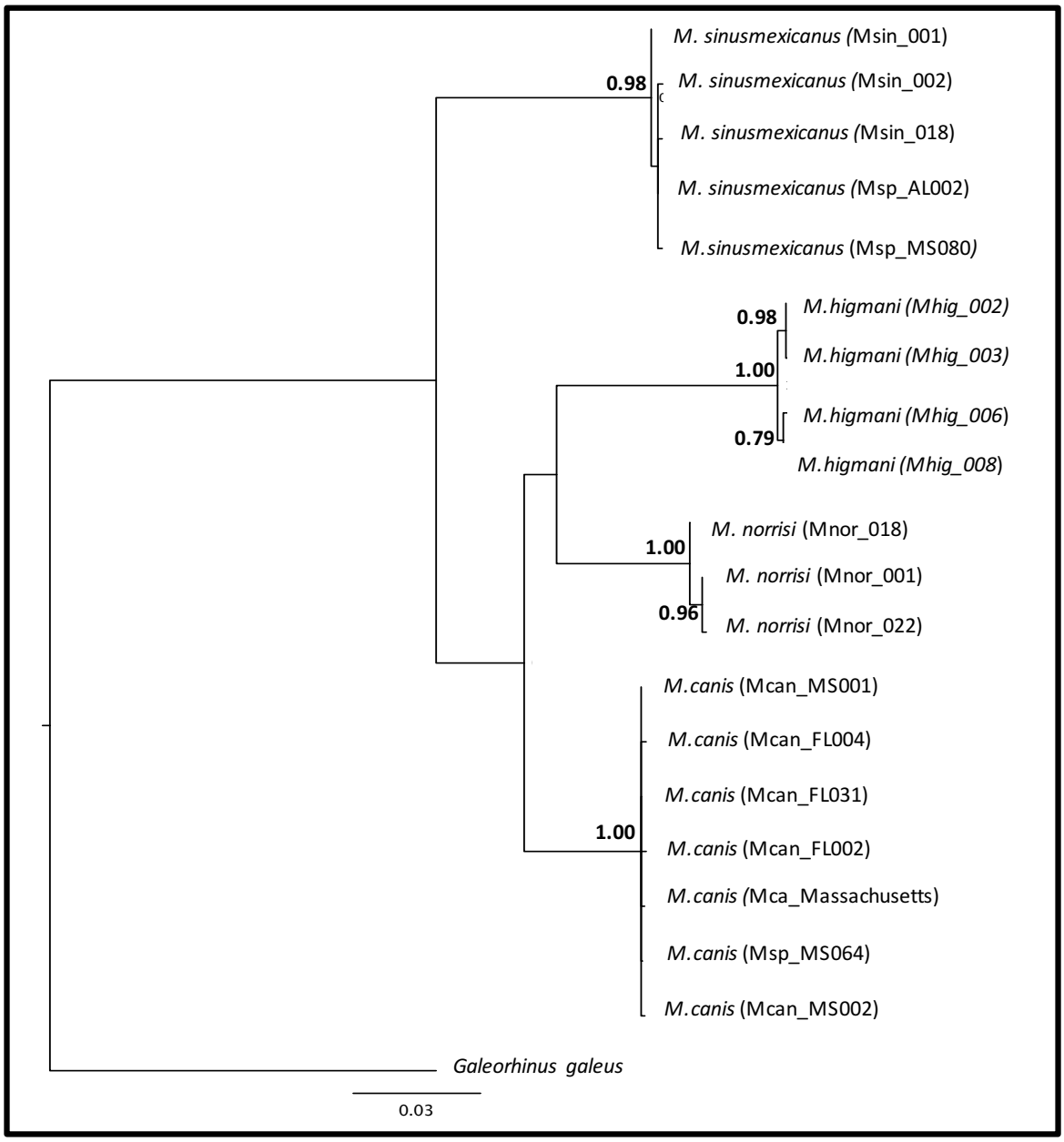

Figure 3.2 Phylogenetic hypothesis (gene tree) inferred from ND-2 sequences of smoothhound sharks from the Gulf of Mexico and from offshore of French Guiana. Numbers on nodes are bootstrap support values; only values greater than 75 % are shown. Bar is number of nucleotide substitutions per site.

relative to the other species. The third clade from the Gulf included several large specimens and was designated tentatively as *M. sinusmexicanus*. Morphological assessment (below) confirmed these tentative species assignments. The fourth clade was assumed to represent *M. higmani* but no voucher material from French Guiana was available for examination. The distribution of mtDNA haplotypes (and GenBank accession numbers) among each of the four species of *Mustelus* is given in Supplementary Table 3.3; the mtDNA haplotype found in each of the 132 individuals assayed is given in Supplementary Table 3.4.

Table 3.3. Distribution of mtDNA haplotypes (and GenBank Accession) among four species of smoothhound sharks (*Mustelus*)

MtDNA Haplotype	*Mustelus canis*	*Mustelus norrisi*	*Mustelus sinusmexicanus*	*Mustelus higmani*	Genbank Accession
#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

Table 3.4 Distribution of mtDNA haplotypes (and GenBank Accession) among specimens of *Mustelus* assayed

Haplotype #	GenBank	Specimen ID
Mustelus canis		
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

Table 3.4 Continued (2)

Haplotype #	GenBank	Specimen ID
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
Mustelus norrisi		
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

Table 3.4 Continued (3)

Haplotype #	GenBank	Specimen ID
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
Mustelus sinusmexicanus		
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
Mustelus higmani		
Haplotype01	KP763703	Mhigmani_006
Haplotype02	KP763704	Mhigmani_001
Haplotype02	KP763704	Mhigmani_002
Haplotype02	KP763704	Mhigmani_008
Haplotype15	KP763717	Mhigmani_003

Results from multi-locus microsatellite assignment were consistent with clades recovered by phylogenetic analysis. Final assignment of individuals to each of three groups (species) was based on 15 microsatellites (Table 3.5) as five microsatellites were either not diagnostic to an individual species or did not amplify across all species.

Table 3.5 Size range (in base pairs) of alleles uncovered from amplifications of 15 microsatellites in three species of *Mustelus*

Microsatellite	Species	Range
*Mca*31	*M. canis*	229-247
	M. norrisi	238
	M. sinusmexicanus	226-238
*Mca*40	*M. canis*	162-170
	M. norrisi	162
	M. sinusmexicanus	160-164
*Mca*44	*M. canis*	169-185
	M. norrisi	169-222
	M. sinusmexicanus	159-222
*Mca*B5	*M. canis*	192-200
	M. norrisi	192-200
	M. sinusmexicanus	196-218
*Mca*B6	*M. canis*	238-250
	M. norrisi	240-256
	M. sinusmexicanus	238-250
*Mca*B22	*M. canis*	141-169
	M. norrisi	151-195
	M. sinusmexicanus	135-171
*Mca*B26	*M. canis*	225-235
	M. norrisi	215-230
	M. sinusmexicanus	220-230
*Mca*B28	*M. canis*	148-150
	M. norrisi	144-146
	M. sinusmexicanus	130-150
*Mca*B35	*M. canis*	186-220
	M. norrisi	200-220
	M. sinusmexicanus	202-214

Table 3.5 Continued (2)

Microsatellite	Species	Range
*Mca*B36	*M. canis*	154-162
	M. norrisi	150-164
	M. sinusmexicanus	152-162
*Mca*B37	*M. canis*	239-255
	M. norrisi	235-245
	M. sinusmexicanus	241-253
*Mca*B40	*M. canis*	166-170
	M. norrisi	167-227
	M. sinusmexicanus	170-215
*Mca*B41	*M. canis*	201
	M. norrisi	199
	M. sinusmexicanus	199
*Mca*25	*M. canis*	260
	M. norrisi	252-260
	M. sinusmexicanus	252-262
*Ma*WS1	*M. canis*	181-193
	M. norrisi	187-195
	M. sinusmexicanus	181-203

The clade containing smoothhounds from French Guiana was not included in STRUCTURE analysis because many microsatellites could not be amplified consistently from fin clips of these specimens. The most likely value of *K* was three (*P* >99%) and assignment of individual smoothhounds was unambiguous; 132 individuals were assigned to the clade designated as *M. canis*, 39 to *M. norrisi*, and 116 to *M. sinusmexicanus*. Of the 287 individuals assayed, 84 (~29%) were either misidentified in the field (61) or identified only as an unknown species of *Mustelus* (23). Results of DAPC analysis (Figure 3.3) 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 confirmed that all three species are divergent genetically from one another.

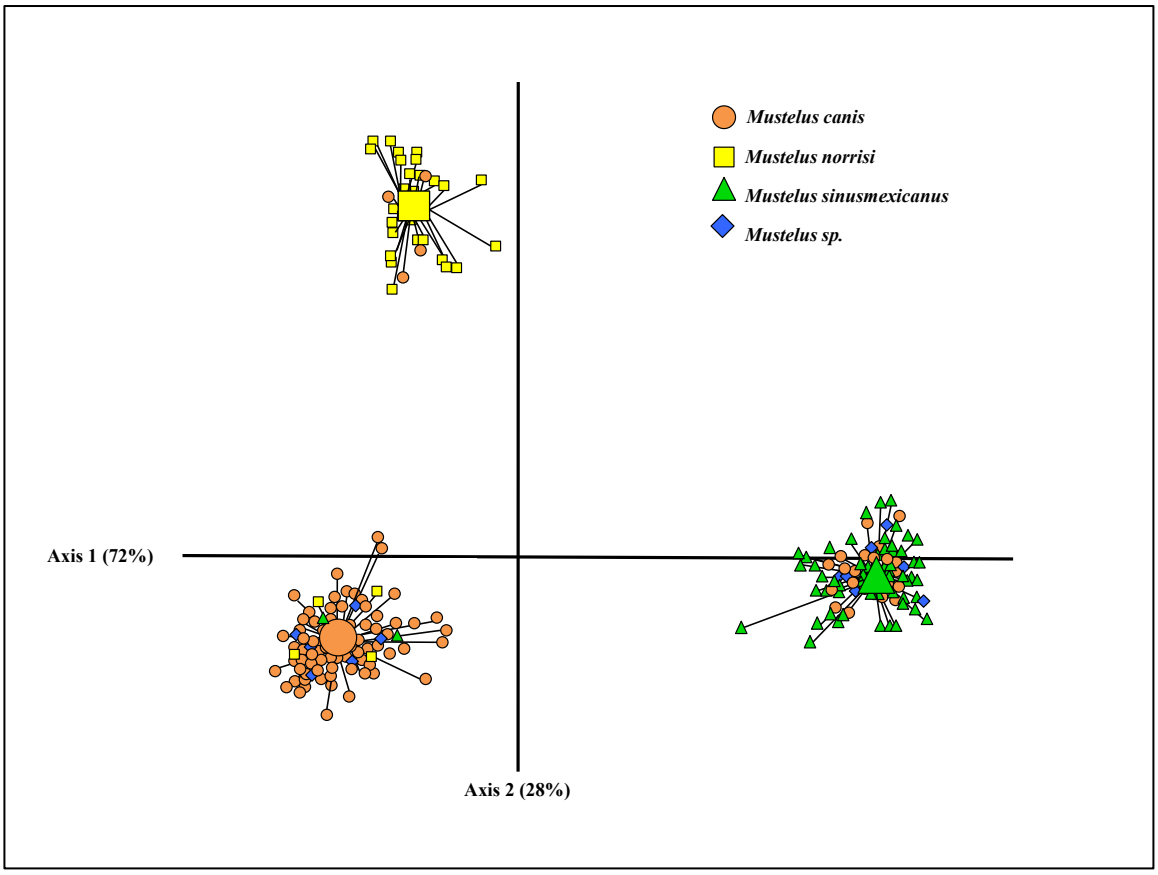

Figure 3.3 Discriminant analysis of principal components (DAPC), based on multilocus microsatellite genotypes, of smoothhound specimens in the northern Gulf of Mexico. Cluster centroids are designated by the largest shape. Individuals that are shaped differently than the centroid were either misidentified or not identified to species in the field. The proportion of variance explained by each axis is given.

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, with type and other curated specimens of each species revealed

macroscopically visible characters that can be used to distinguish among adult specimens of each species (Figures 3.5, 3.6 and 3.7). *Mustelus canis* when laid flat is identified by the relatively straight posterior margins of the pelvic and pectoral fins and by nasal flaps that are medially expanded. Adult *M. norrisi* are identified by an acutely pointed, posteriorly directed lower lobe of the caudal fin (as noted by Bigelow and Shroeder (1948) and Heemstra (1997)). In addition, adult males of *M. norrisi* are identified by the presence of calcified claspers in individuals smaller than 65 cm total length (Heemstra 1997). *Mustelus sinusmexicanus* is identified by very long, upper labial furrows that extend to a perpendicular line even with the symphysis of the lower jaw, by biserial rows of ampullae of Lorenzini (the ventral group of outer buccal tubules *sensu* Chu and Wen 1979) far posterior to the upper labial furrows and extending to the first gill slit, and by nasal flaps that are narrow with an acute posterior margin. The ampullae in *M. canis* and *M. norrisi* are posterior to the upper labial furrows are uniserial and the nasal flaps are medially expanded with relatively straight posterior margins. A dichotomous key can be found in Appendix I.

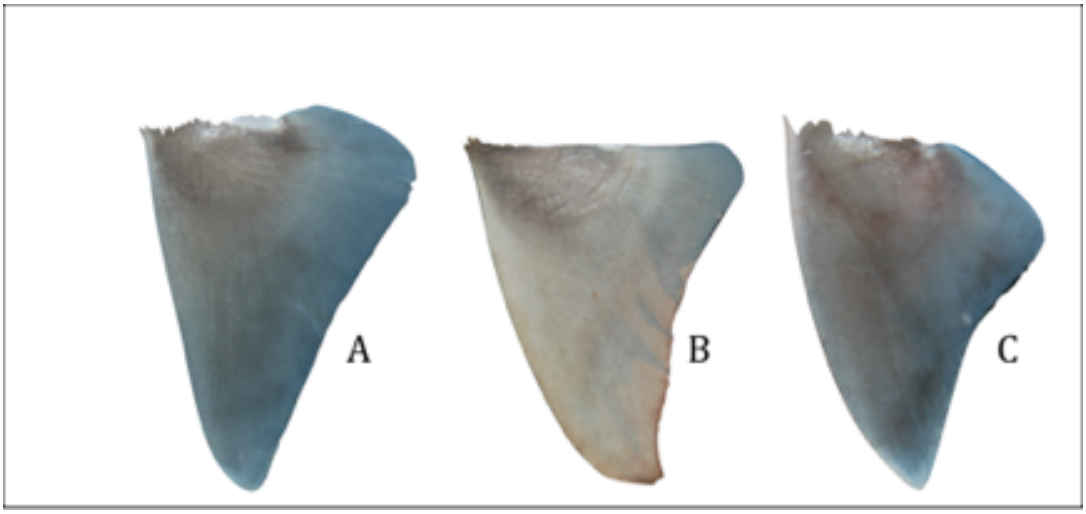

Figure 3.4 Pectoral fin comparison among species of *Mustelus* in the northern Gulf of Mexico; insertion to body is located at the top left corner of each fin; posterior margin of pectoral fin is the rightmost edge, nearest to letter. **A** – pectoral fin of *M. canis,* with a nearly straight posterior margin; **B** – pectoral fin of *M. sinusmexicanus* with a falcate posterior margin; **C** – pectoral fin of *M. norrisi* with a falcate posterior margin.

Figure 3.5 Caudal fin comparison among specimens of *Mustelus* in the northern Gulf of Mexico. **A** – lower lobe of caudal fin in *M. norrisi* is slightly falcate with an acute tip directed backwards; **B** – lower lobe of caudal fin in *M. canis* is nearly straight with a rounded tip; **C** – lower lobe of caudal fin in *M. sinusmexicanus* is falcate with a rounded tip, angled backwards.

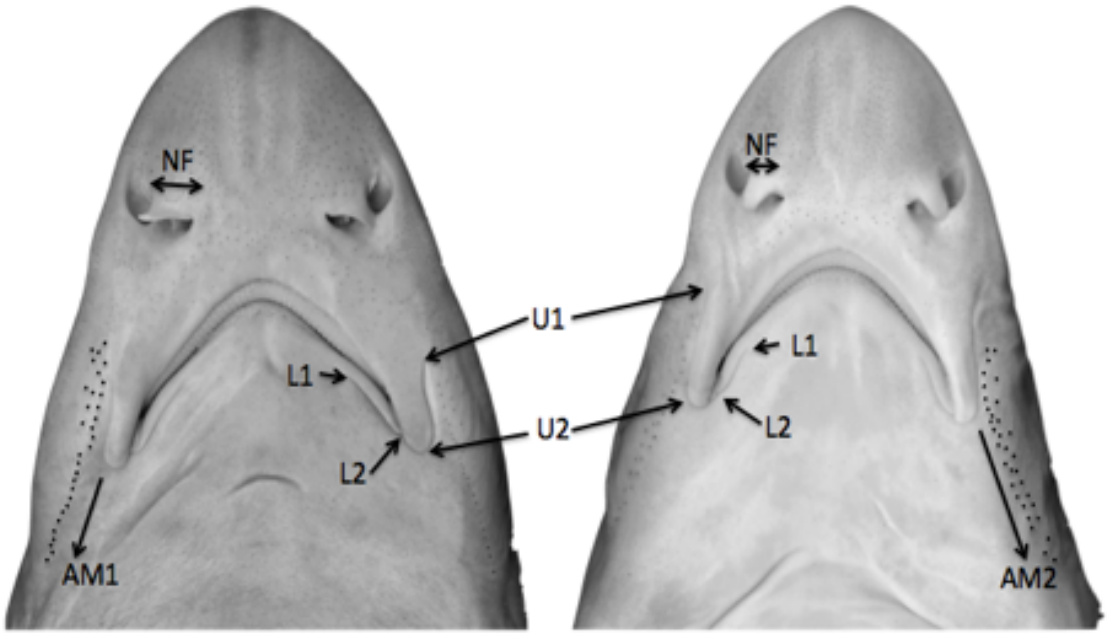

Figure 3.6 Differences on the ventral surface of the head among species of *Mustelus* in the U.S. Gulf of Mexico. The specimen on the left is *M. canis*; the specimen on the right is *M. sinusmexicanus*. **NF** represents the anterior nasal flaps (medially expanded in *M. canis*); **L1** is the anterior bound of the lower labial furrow, **L2** is the posterior bound of the lower labial furrow; **U1** is the anterior bound of the upper labial furrow, **U2** is the posterior bound of the upper labial furrow. **AM** represents ampullae of Lorenzini directly posterior to upper labial furrows (i.e., ventral group of outer buccal tubules): **AM1** shows one row of ampullae (*M. canis* and *M. norrisi*), while **AM2** shows two rows of ampullae (*M. sinusmexicanus*). Ampullae and Ampullae and posterior margin of nasal flaps were darkened electronically for emphasis.

The first two dimensions of multi-factorial analysis (MFA) explained 75% of the variance and revealed that the distribution of individuals of the three species was not homogenous along the two axes (Figure 3.7); *M. norissi* was found primarily in shallow waters, while *M. canis* was found in the deepest waters. Estimated mean depth of capture (± S.E.) for all three species (based on GLHT) followed the same pattern and differed significantly in pairwise comparisons with estimated mean depth (± S.E.) of all sampling events (138.13 ± 8.64 m): *M. norissi* (15.80 ± 7.44 m, $t = -5.471$, $P < 0.001$), *M. sinusmexicanus* (112.01 ± 6.51 m, $t = -2.64$, $P = 0.024$), and *M. canis* (179.74 ±

13.36 m, $t = 5.86$, $P < 0.001$). Captures of *M. norrisi* were primarily in the eastern Gulf (also noted by Heemstra 1977), whereas captures of *M. canis* and M. *sinusmexicanu*s occurred across the sampling area (Figure 3.1). Estimated mean month and longitude of capture of both *M. norissi* and *M. sinusmexicanus* differed significantly from the estimated mean month (mid-July) and mean longitude (−88.60°) of all sampling events. Mean month and longitude of capture for *M. norissi* was mid-May ($t = -3.20$, $P = 0.005$) and −85.60 ($t = 3.43$, $P = 0.002$), respectively; whereas mean month and longitude of capture for *M. sinusmexicanus* was early August ($t = 2.63$, $P = 0.024$) and –89. 63° ($t = -2.66$, $P = 0.022$), respectively. Both estimated mean month and mean longitude for *M. canis* did not differ significantly from the estimated mean of all sampling events.

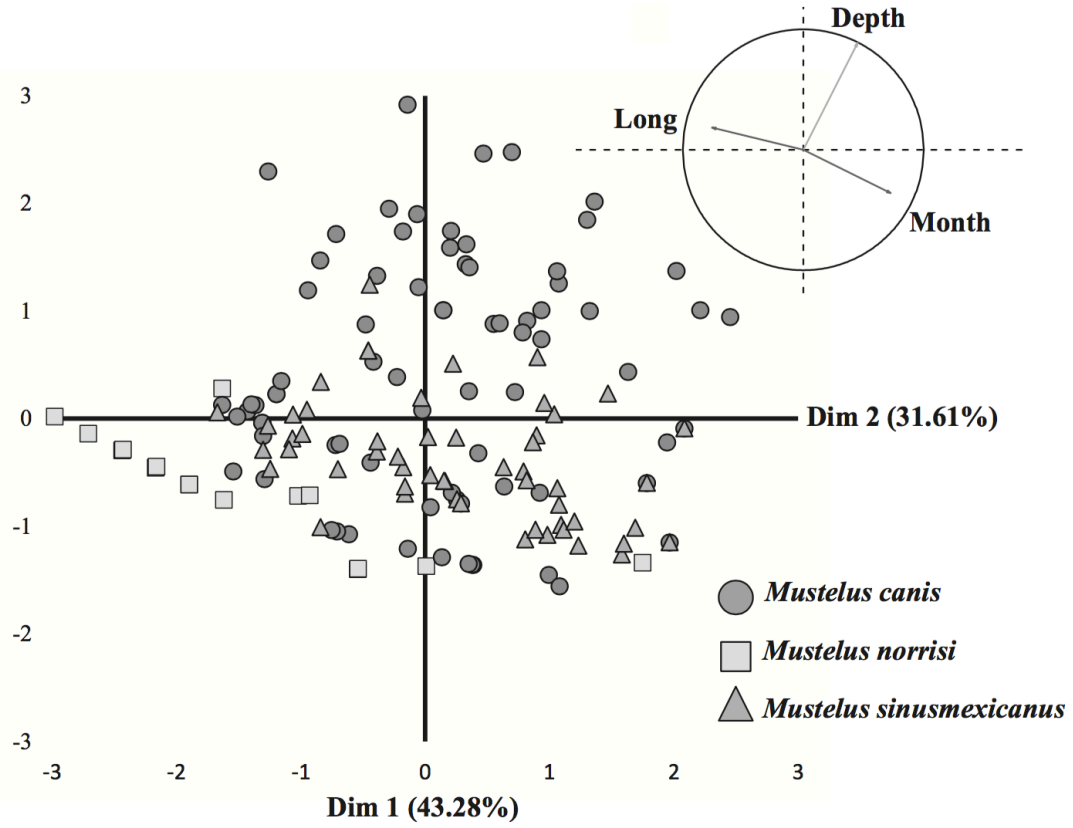

Figure 3.7 Multiple factor analysis of depth, month, and longitude of all sampling events of species of *Mustelus*. Species identity is overlaid on each individual data point: circles (*M. canis*), squares (*M. norrisi*), and triangles (*M. sinusmexicanis*). Inset indicates directionality of each factor on the MFA plane.

Discussion

Genetic data (mtDNA sequences and microsatellite genotypes) obtained in this study are consistent with the occurrence of three, genetically distinct taxonomic units (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 employed to reduce misidentifications during routine in-the-field surveys, allowing for assessments of 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 to distinguish among neonates or juveniles of each 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 *M. norrisi* and *M. canis* are genetically distinct and not the same species.

Multi-factorial analysis and homogeneity tests of species-specific means versus grand means of depth, longitude, and month of capture of genetically identified smoothhounds revealed differences among the three species in preferred depth and between *M. norrisi* and *M. sinusmexicanus* in average longitude and month of capture. *Mustelus canis* tends to prefer deeper waters (range 64-408 m) than *M. sinusmexicanus* (range 51-233 m), while *M. norrisi* inhabits relatively shallow waters (1-92 m). Heemstra (1997) reported similar differences in depth of capture of *M. norrisi* and *M. sinusmexicanus*; however, the maximum depth found in this study for *M. canis* (408 m) is greater than the depth (360 m) previously reported for the species (Heemstra 1977). The occurrence of *M. canis* in deeper waters in the Gulf may be due in part to preference/tolerance for colder temperatures. This is consistent with the behavior of *M. canis* along the east coast of the United States where the species migrates from the

Carolina coast northward to colder waters along the New England coast during the summer months and returns southward during the winter months (Castro 2011; SEDAR 2014). Captures of *M. norrisi* were concentrated in the eastern Gulf, whereas captures of *M. sinusmexicanus* tended to be further to the west. There also was an apparent seasonal difference in capture between *M. norrisi* (late spring) and *M. sinusmexicanus* (late summer).

Sampling localities of the three species across the northern Gulf in this study were more or less consistent with those reported by Heemstra (1997) although we did find several individuals of *M. sinusmexicanus* farther to the east than reported in Heemstra (1997). Captures of *M. norrisi* 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 off the lower coast of Texas. However, because sampling in our study 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 temporal and spatial differences in distribution of all three species.

No individuals of *M. higmani* were recovered in the Gulf during the study. The lone specimen of *M. higmani* 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 ~800 meters deeper than any other known records for the species (Heemstra 1973, 1997). Extensive long-line sampling of DeSoto Canyon, 320 stations

between 200 – 2,000 m, occurred during this study and only *M. canis* was captured from depths greater than 400 m.

Conclusions

Reciprocally monophyletic clades recovered in phylogenetic analysis of mtDNA haplotypes, distinct genetic clusters based nuclear-encoded microsatellites, and distinctive characters in external morphology, demonstrated occurrence of three of three genetically distinct lineages of smoothhound sharks in the Gulf, identified as *M. canis*, *M. norrisi*, and *M. sinusmexicanus*. The three species co-occur in the Gulf but appear to have different depth preferences and perhaps spatial/temporal distributions. These results provide fisheries scientists with a simple morphological key to distinguish among species in the field and also suggest 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 is warranted.

CHAPTER IV

POPULATION GENETIC STRUCTURE OF THE DUSKY SMOOTHHOUND SHARK, *MUSTEUS CANIS,* IN U.S. WATERS

Synopsis

The dusky smoothhound shark, *Mustelus canis,* is a small, demersal species that inhabits continental and insular shelves in the western Atlantic Ocean. Tagging data suggest that this species may undertake seasonal migrations along the eastern seaboard of the United States. We assayed the entire mitochondrially encoded NADH-2 gene (1047 bp) and 15 nuclear-encoded microsatellites from individuals collected along the east coast of the United States and from the northern Gulf of Mexico to estimate the degree of population subdivision. Mitochondrial haplotype diversity and nucleotide diversity were low relative to some other shark species. Similarly, there were low levels of diversity detected in comparisons of microsatellites. Comparisons of pairwise F_{ST} between localities and results of Analysis of Molecular Variance (AMOVA) indicate that there is significant genetic subdivision between the Atlantic and northern Gulf of Mexico and also between the localities in the eastern and western Gulf.

Introduction

The dusky smoothhound shark, *Mustelus canis,* is widely distributed in waters of the western Atlantic Ocean, from Canada to the state of Florida, through the northern

Gulf of Mexico, and from southern Brazil through Argentina (Compagno 2005). *Mustelus canis* is displaced by an insular form (the nominal subspecies *Mustelus canis insularis*, in Cuba, Jamaica, Barbados, Bermuda, and the Bahamas (Heemstra 1997). Although *M. canis* is commonly encountered along the Atlantic coast of the United States (herein Atlantic), little is known about its movement patterns or about fisheries stock structure in this region. Bigelow and Schroeder (1948) hypothesized that *M. canis* is divided into discrete stocks in the Atlantic: a northern stock that migrates during the summer months from wintering grounds in Virginia and the Carolinas to the waters off New York, New Jersey, and southern New England, and another stock that presumably migrates offshore in the winter months. Their hypothesis is supported by limited tagging data in that individuals caught and tagged in New England were re-captured in the Carolinas later in the same year (Kohler et al. 2014). Little is known about *Mustelus canis* in the northern Gulf of Mexico (herein Gulf).

The dusky smoothhound shark (*M. canis*) supports commercial and recreational fisheries along the U.S. Atlantic coast (SEDAR 2015). From 1990-2012, commercial landings of smoothhound sharks in the Atlantic averaged 1,897,927 metric tons (mt), peaking at 3,991,700 mt in 2010 (SEDAR 2015). Recreational landings during the same period averaged 733,680 mt, peaking at 1,997,431 mt in 2006 (SEDAR 2015). Conversely, the total catch in the northern Gulf of Mexico (herein Gulf) peaked at 50,000 lbs (22.680 mt) in 1989 and hasn't risen above 1000 lbs (.453 mt) since 1991 (SEDAR 2015). Currently, smoothhound management in the US varies by state, with most Atlantic states following the Atlantic States Fisheries Management Council

(ASFMC) regulations. The ASFMC regulations dictate an annual quota, which is then divided into state shares. Within the recreational fishery, each angler may keep one smoothhound per trip. Smoothhounds are not federally managed, but the fishery soon will be managed, because the South East Data Assessment and Review (SEDAR) completed an assessment of the smoothhound shark fishery in 2015.

There were two major decisions that were made based on the SEDAR assessment. The first decision is that *Mustelus canis* will be treated as two separate stocks; the east coast of the United States (Atlantic) will be treated as a single stock and the northern Gulf of Mexico (Gulf) will be treated as the second stock (SEDAR 2015). The second decision based on the SEDAR is that the smoothhounds in the Gulf of Mexico will be managed as a species complex, inclusive of *M. canis, M. norrisi,* and *M. sinusmexicanus*. The first decision (to manage *M. canis* as two separate stocks) was based on several lines of evidence including tagging data, which though limited, showed no movement of smoothhounds between the Atlantic and Gulf (SEDAR 2015). Initial analyses from this study also showed that there were differences in mitochondrial haplotype distributions between *M. canis* in the Atlantic and in the Gulf. In addition, assessments of life history data indicate that there may be differences in life history characteristics between *M. canis* in the Gulf and *M. canis* in the Atlantic (SEDAR 2015). The decision to manage the Atlantic and Gulf as two separate stocks is in part, due to the fact that there is currently a directed fishery for *M. canis* in the Atlantic and there is not presently a directed fishery for smoothhounds in the Gulf of Mexico (SEDAR 2015). Whereas there is only one species of smoothhound shark in the Atlantic (*M. canis),* there

are three species present in the Gulf (*M. canis, M. norrisi,* and *M. sinusmexicanus;* Giresi et al. 2015). The decision to treat smoothhound sharks in the Gulf as a species complex is due largely in part to the fact that the species are difficult to distinguish from each other morphologically and at the time of the assessment, there was no consistent key to distinguish among the species. The National Marine Fisheries Service recognizes the need for species-specific life history parameters and species-specific landings data, and will re-visit their management plan after this information is more readily available (SEDAR 2015). Presently, smoothhounds are not considered overfished or experiencing overfishing in either the Atlantic or in the Gulf (SEDAR 2015), but baseline information on genetic population structure for all three species is essential for future population monitoring.

Investigating the degree of population structure of a species is expected to provide insights into the evolution and behavior of that species, as well as providing guidance for management plans that are in line with the biological sustainability of that species. The expectation is that while many fishes, including elasmobranchs, have wide distributions and are potentially capable of long migrations, genetic exchange may not be ubiquitous and barriers to gene flow may occur on relatively small scales. Evidence of genetic subdivision has been documented on relatively small scales for several elasmobranchs in the western Atlantic Ocean, including the blacktip shark, *Carcharhinus limbatus* (Keeney et al. 2005), bull shark, *Carcharhinus leucas* (Karl et al. 2011); nurse shark, *Ginglyostoma cirratum* (Karl et al. 2012); scalloped hammerhead shark, *Sphryna lewini* (Duncan et al. 2006); lemon shark *Negaprion brevirostris* (Feldheim et al. 2001; Ashe et

al 2015); and *Carcharhinus acronotus* (Portnoy et al. 2014). It is important to note that in most of the studies, evidence of population structure is primarily from mtDNA markers, whereas microsatellites provide low or no evidence of genetic structure in many of these studies. Authors of these studies often attribute the genetic structure of mtDNA to female philopatry. Species may also have limited exchange between the Atlantic and Gulf because of The Loop Current (Wiseman and Sturges 1999) through the Florida Straits. The deep water and strong current present a barrier for a multitude of taxa including invertebrates (Lee et al. 1994, Wicksten and Packard 2005), and for other small sharks (*Carcharhinus acronotus*, Portnoy et al. 2014; *Sphyrna tiburo,* Portnoy *et al.* 2015 and *C. isodon*).

The primary goal of this study was to assess genetic population structure of *Mustelus canis* between and among geographic localities along the eastern seaboard of the United States and from the northern Gulf of Mexico. The study tests the null hypothesis that *Mustelus canis* along the eastern seaboard of the United States and from the northern Gulf of Mexico represent a single genetically panmictic population. A total of 15 nuclear microsatellite loci and sequences of the (1047-bp) mitochondrially encoded NADH-2 gene were scored and employed for this test.

Materials and Methods

Sampling and Locality Designation

A total of 504 individuals were sampled between 2010 and 2013 from localities in

the Atlantic and Gulf. However, only individuals that were collected between the months of April and September were included in the final dataset. In total, 377 individuals were included in the final dataset. Biologically, this makes sense, because reproductive stocks are separated during summer months; it is known that females give birth inshore during the summer months and that males wait to mate immediately after parturition. During the winter months, it is possible that reproductive stocks may mix. In the Atlantic, the sampling was mostly discrete, so separating sample localities for analysis was straightforward, whereas, in the Gulf, the sampling was continuous and thus separating samples into units for genetic analyses was more complicated. Individuals were grouped for analysis as follows; Massachusetts, MA (n=111); Delaware Bay, DB (n=140); South Carolina, SC (n=33); the west coast of Florida, FL (n=47); central Gulf (Alabama and Mississippi), MS (n=19), and western Gulf (Louisiana and Texas), TX (n=23). Sampling localities are shown in Figure 4.1. Sample collection data is in Appendix Table 4.1.

DNA Extraction

Tissues (fin clips, ~1 cm^2) were taken from the trailing edge of the first dorsal, left pelvic fin, or the sub-terminal notch of the caudal fin of each individual. Tissues were fixed in 20% DMSO buffer (Seutin et al. 1991) or 95% non-denatured ethanol and stored at room temperature. Total DNA was extracted using a modified chelex resin (Bio-rad®) extraction protocol (Estoup et al. 1996) or a phenol-chloroform-isoamyl alcohol protocol

(Sambrook et al. 1989).

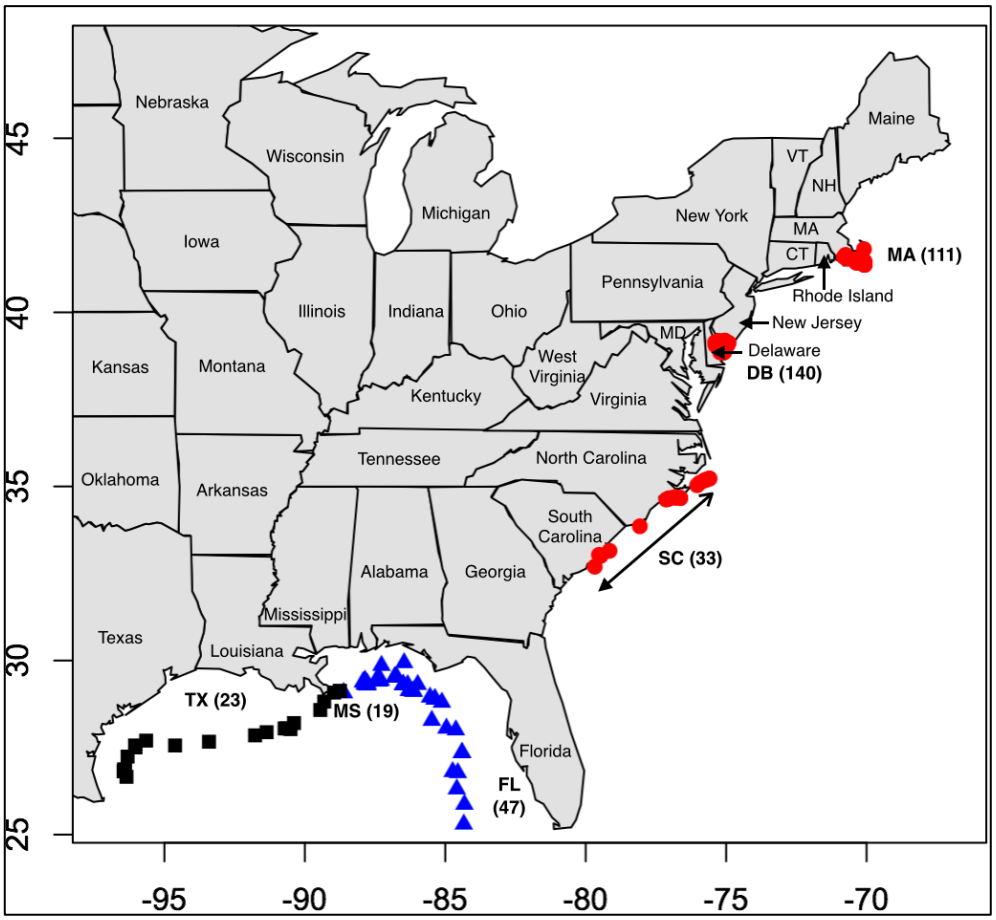

Figure 4.1 Map of sample localities of *Mustelus canis* in U.S. waters. Sampling locations are indicated with number of samples collected from each region in parentheses. Red circles represent locations where individuals are from the Atlantic (MA, DB, SC), blue triangles represent locations where individuals were part of the eastern/central Gulf (FL, MS), and black squares represent sampling locations where individuals that were part of the western Gulf (TX).

Microsatellites

Genotypes at 15 nuclear-encoded microsatellites were acquired from all individuals sampled. PCR primers and protocols are given in Giresi *et al* (2011). The forward

primer was fluorescently labeled with either 6-FAM, HEX, or NED (Dye Set D; Applied Biosystems). Amplicons were electrophoresed on 6% polyacrylamide gels, using an ABI Prism 377 automated sequencer (Applied Biosystems). Each lane included a 400 base-pair size standard (Genescan 400HD ROX™, Applied Biosystems). Allele sizing and scoring was conducted manually, using Genescan v. 3.1.2 (Applied Biosystems) and Genotyper v. 2.5 (Perkin Elmer).

Hardy-Weinberg equilibrium (HWE) was tested for each microsatellite in each sample locality using GENEPOP v.4.1 (Raymond & Rousset 1995; Rousset 2008; http://kimura.univ-montp2.fr/~rousset/Genepop.htm); significance was assessed using exact tests with 1,000 batches and 10,000 iterations per batch. Deviations from genotypic equilibrium (pairs of microsatellites) were assessed with exact tests, using the Markov chain approach in GENEPOP with 5,000 dememorizations, 500 batches, and 5,000 iterations per batch (Guo and Thompson 1992; Raymond and Rousset 1995). Significance levels for multiple tests carried out simultaneously were adjusted using sequential Bonferroni correction (Rice 1989). Number of alleles, rareified allelic richness (El Mousadik & Petit 1996), expected heterozygosity (unbiased gene diversity, Nei 1987), the inbreeding coefficient (F_{is}), and were estimated using HIERFSTAT (Goudet 2005). Wilcoxon signed-rank tests, implemented in JMP PRO v11.2.0 (SAS Institute Inc.), were used to test for homogeneity of gene diversity and allelic richness between all pairs of localities.

Homogeneity in allele and genotype distributions for microsatellite data among samples was tested using a single-level, analysis of molecular variance (AMOVA),

implemented in ARLEQUIN (Schneider et al. 2000; Excoffier & Lischer 2010). Pairwise F_{ST} values between samples were estimated using GENODIVE (Meirmans, and Van Tienderen, 2004). Significance of pairwise F_{ST} values was assessed ($\alpha < 0.05$) using a Markov chain approach with 50,000 permutations; correction for multiple tests followed Benjamini and Hochberg (1995). To examine hierarchical population structure, a second AMOVA was run in ARLEQUIN with sample groupings determined by similarity of values based on visual inspection of pairwise F_{ST} results.

A Bayesian clustering approach implemented in STRUCTURE 2.3.4 (Pritchard et al. 2000; Falush et al. 2007) was used to further evaluate population structure using the no-admixture model with correlated allele frequencies. Five replicates of *K* groups (K = 1-6) were run using a burn-in of 500,000 steps followed by a run of 1,000,000 steps. The number of clusters was selected by evaluating likelihood, L(*K*) in STRUCTURE HARVESTER (Earl and von Holdt, 2012).

mtDNA

The complete mitochondrial gene, NADH-2 (ND2, 1047bp), was amplified using the primers *Mus*ND2F: 5'-CCA TAC CCC AAC CAT GTG GTT-3' and *Mus*ND2R: 5'-GCT TTG AAG GCT TTT GGT CTG-3' (Giresi et al. 2015) from a subset of individuals from each sample locality. Thirty microliter reactions containing 1 X reaction buffer (pH 8.5), 2.4 mM $MgCl_2$, 2.4 mM dNTPs, 1.5 uM of each primer, 0.5 U⁄μL *Taq* polymerase (GoTaq Flexi DNA Polymerase, Promega), and 3 uL DNA

template. Reaction conditions included an initial denaturation at 95°C for 3 min, followed by 40 cycles of 95°C for 30 sec, 60°C for 1 min and 72°C for 1 min, followed by a final extension of 72°C for 10 min. Products were electrophoresed on 2.0% agarose gels, extracted and purified with a QIAquick Gel Extraction kit (Qiagen). PCR products were sent to Beckman Coulter (http:/beckmangenomics.com/) for bi-directional sequencing. Sequence chromatograms were corrected by eye and aligned using Sequencher v.4.8 (Gene Codes Corp.). Unique haplotypes were identified using DNASP (Rozas et al. 2003) and will be deposited in GenBank.

Genetic diversity within sample localities was estimated as the number of haplotypes, haplotype diversity (h), and nucleotide diversity (π), using DnaSP (Rozas et al. 2003). Homogeneity of haplotype distributions among sample localities was tested using single-level AMOVA, implemented in ARLEQUIN. Pairwise Φ_{ST} values also were estimated using ARLEQUIN, with significance determined ($\alpha < 0.05$) using a Markov chain approach with 10,000 permutations. Correction for multiple tests followed Benjmaini and Hochberg (1995). A hierarchical AMOVA was run in ARLEQUIN; the sample groupings were the same as described for microsatellites. Relationships among mtDNA haplotypes were visualized in a minimum-spanning network constructed using the median-joining algorithm in NETWORK (Bandelt et al. 1999). Mantel tests (Smouse et al. 1986) were implemented in ARLEQUIN with 10,000 permutations, to test whether divergence estimates between genetic marker types were correlated.

Results

Microsatellites

A significant departure from Hardy-Weinberg expectations was detected at four of six *a priori* geographic samples for *Cis*163; this locus was removed from subsequent analyses. Five additional loci also deviated from the expectations of Hardy-Weinberg equilibrium prior to Bonferroni correction but only for one sample each; no locus sample pairs were significant after correction for multiple tests. Sequential Bonferroni was run for each sample separately. In total, 25 of the pairwise tests of genotypic equilibrium were significant before sequential Bonferroni correction, but none of the pairwise tests remained significant after correction. Two sets of summary statistics are presented. In Appendix Table 4.2, summary statistics are presented for five localities (MA, DB, SC, eastern Gulf; inclusive of FL and MS, and west Gulf, TX) and for the Atlantic (inclusive of MA, DB, SC) and Gulf (east and west Gulf) separately.

Single-level AMOVA, based on microsatellites indicated that there was significant heterogeneity among geographic samples ($F_{ST} = 0.019$, $P < 0.001$). Pairwise estimates of F_{ST} (microsatellites) were significant, both before and after correction for multiple tests, between all Atlantic and Gulf samples and also between FL and TX (Table 4.1). Individuals from Mobile Bay to western Florida were genetically indistinguishable from each other.

Table 4.1: Pairwise Population Differentiation among *Mustelus canis* from locations in the Atlantic Ocean and Gulf of Mexico: Pairwise F_{ST} (microsatellites) above diagonal; pairwise *f* (mtDNA) below diagonal. Significant values of pairwise comparisons are in bold.

	MA	DB	SC	FL	MS	TX
MA	---	0.001	0.004	**0.028**	**0.022**	**0.019**
DB	0.008	---	0.002	**0.021**	**0.016**	**0.014**
SC	0.065	0.045	---	**0.032**	**0.017**	**0.024**
FL	**0.05**	**0.065**	**0.105**	---	-0.001	**0.015**
MS	0.016	0.024	**0.044**	-0.091	---	0.011
TX	-0.002	0.027	**0.094**	0.004	-0.021	---

Hierarchical AMOVA with groupings of Atlantic and Gulf indicated significant heterogeneity between the ocean basins (F_{CT} = 0.018, P < 0.001, Table 4.2). There was evidence of genetic heterogeneity within ocean basins as well (F_{SC} = 0.004, P = 0.007).

Table 4.2: Results of hierarchical AMOVA for the smoothhound shark, *Mustelus canis,* based on microsatellites and mtDNA. Regions are Atlantic (MA, DB, and SC) and Gulf (FL, MS, TX); df = degrees of freedom; SS = sum of squares; VC = variance components; %V = proportion of variance; *F* = Fixation Index*; p-values* = probability that *F* = 0.

Microsatellites		***F***	**Df**	**SS**	**VC**	**%V**	***p*-values**
Between Ocean Basins	F_{CT}	0.018	1	19.48	0.055	1.80	0.000
Among Localities within Ocean Basins	F_{SC}	0.004	3	13.49	0.010	0.37	0.007
Among Individuals within Localities			372	2224.10	2.971	97.83	
mtDNA		***F***	**Df**	**SS**	**VC**	**%V**	***p*-values**
Between Ocean Basins	Φ_{CT}	0.042	1	1.37	0.018	4.23	0.000
Among Localities within Ocean Basins	Φ_{SC}	0.028	4	2.27	0.012	2.71	0.078
Among Individuals within Localities			84	33.93	0.404	93.06	

Consistent with the results of hierarchical AMOVA, the results from Bayesian clustering analyses also indicated that there were genetic differences between the Atlantic and Gulf. The results showed that the most likely value of *K* was two,

identified by evaluating L(*K*);see Figure 4.2a; the Atlantic samples were identified as one cluster and Gulf samples identified as the other cluster (Figure 4.2b). There was no evidence of structuring within ocean basins. When STRUCTURE was run with the Atlantic Gulf separately, the most likely value of *K* also was one.

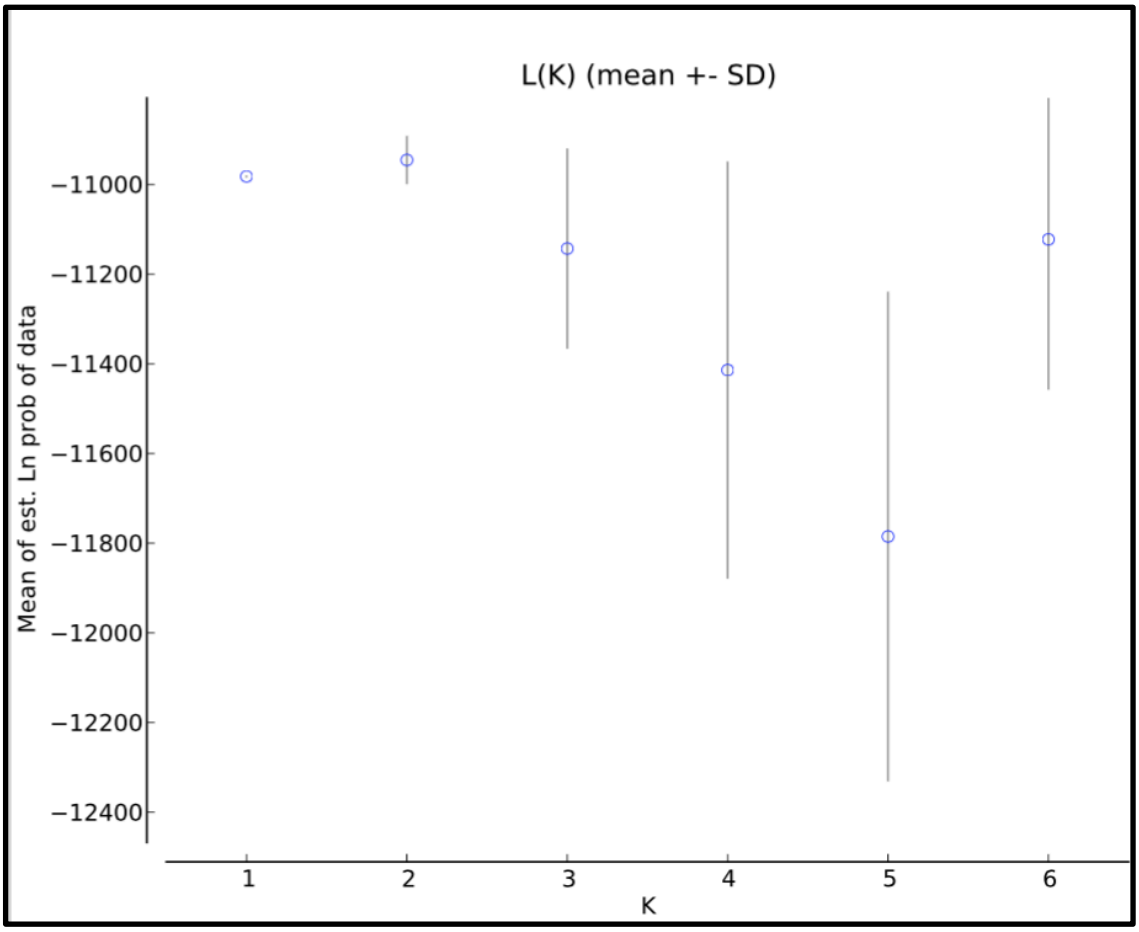

Figure 4.2a Plot of mean ln(PD) for all runs of *K*=1 through *K*=6 for *M. canis* in the Atlantic and northern Gulf of Mexico. *K* = 2 is the most likely value of *K*.

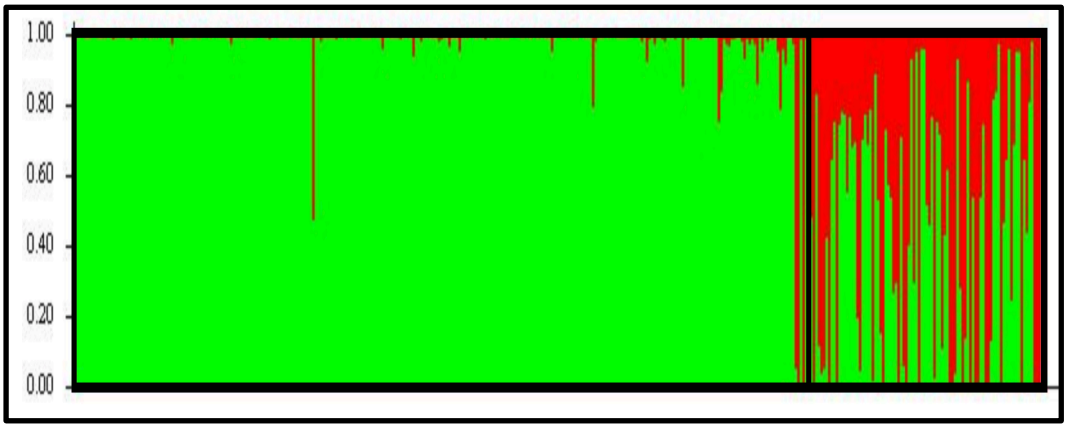

Figure 4.2b Visualization of K=2 from Bayesian clustering analysis in STRUCTURE. Green: individuals from cluster one; primarily from the Atlantic (MA, DB, SC). Red: individuals from cluster two, primarily from localities in the Gulf (FL, MS, TX). The y-axis represents the posterior probability of assignment to a cluster; individuals are on the x-axis.

mtDNA

Summary statistics for microsatellites are presented in Table 4.3. There were 16 polymorphic sites, resulting in a total of 17 haplotypes sampled across all localities, with 63% of the individuals sampled sharing a single haplotype. Estimates of h ranged from 0.395 in MA to 0.905 in SC; while estimates of π ranged from 0.0004 in TX to 0.0018 in SC. The distribution of haplotypes within each sample is presented in Table 4.4.

Table 4.3: Summary statistics for the mtDNA haplotypes from *Mustelus canis* throughout the Atlantic and northern Gulf of Mexico. MA, Massachusetts; DB, Delaware Bay; NC, North Carolina; SC, South Carolina; WFL Western Florida; MS, Mississippi; TX; Overall, all samples combined.

	MA	DB	SC	FL	MS	TX	Overall
Sample size (N)	23	26	7	18	8	13	95
Number of haplotypes (H)	6	8	5	5	4	4	17
Nucleon diversity (H_D)	0.395	0.655	0.905	0.673	0.643	0.423	0.596
Nucleotide diversity (π_D)	0.0005	0.0009	0.0018	0.0008	0.0007	0.0004	0.0008

Table 4.4: Distribution of mtDNA haplotypes among *Mustelus canis* from locations in Atlantic Ocean and northern Gulf of Mexico. Accession numbers that are present in table currently are from Giresi et al. 2015. Blank accession numbers will be submitted for final publication in a scientific journal when the manuscript is ready for submission.

mtDNA haplotype	MA	DB	SC	FL	MS	TX	GenBank Assession
#1	18	15	2	10	5	10	KP763708
#2	1						
#3	1	1	2				
#4	1		1				
#5	1	1					
#6	1						
#7		1					
#8		2	1				
#9		1					
#10		1					
#11		1					
#12			1				
#13				1	2		KP763712
#14				2	1	1	KP763709
#15				1		1	KP763714
#16				3	1		KP763710
#17						1	KP763711

The single-level AMOVA, based on mtDNA sequence data also was significant (Φ_{ST} = 0.033, P = 0.014). Estimates of pairwise Φ_{ST} (mtDNA) were significant between SC and all Gulf samples, and between all comparisons between FL and all Atlantic localities. After correction for multiple tests, only the estimate of pairwise Φ_{ST} between FL and DB was significant (Table 4.1). Hierarchical AMOVA using the same groupings as used in microsatellite analysis (Atlantic and Gulf) indicated significant heterogeneity between the ocean basins (Table 4.2). The minimum spanning network showed no clear evidence of partitioning of haplotypes among the samples (Figure 4.3).

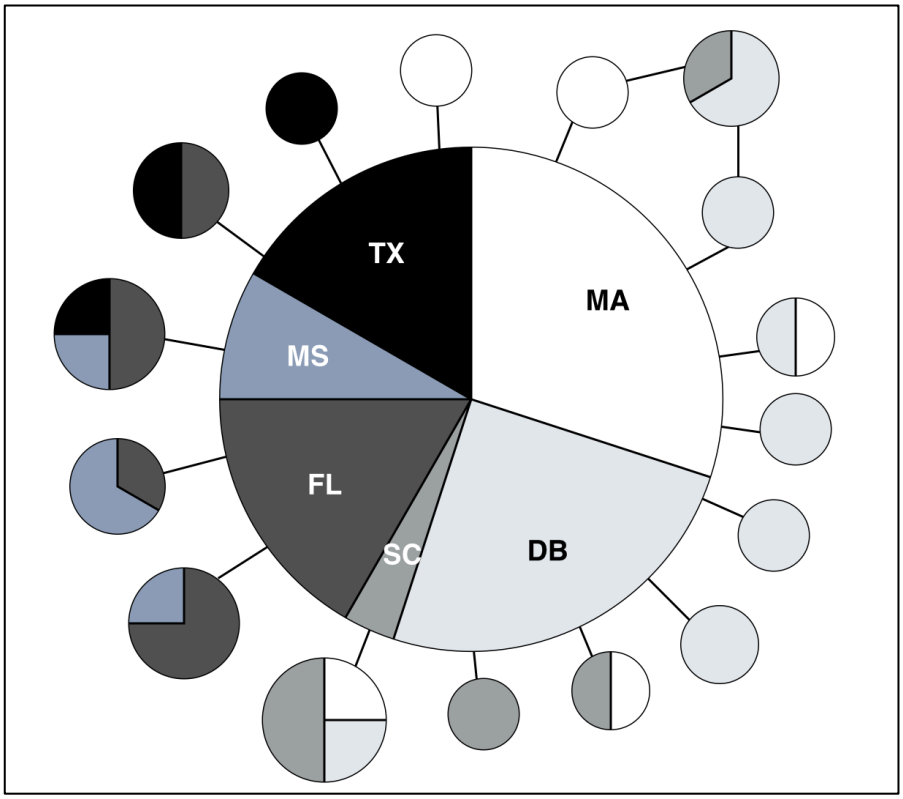

Figure 4.3 Minimum spanning network of 17 mtDNA haplotypes from 95 individuals of *Mustelus canis*. Size of each circle is representative of the number of individuals who share a given haplotype. The size of the circle is representative of the number of individuals that share each haplotype.

Of the 16 satellite haplotypes, 15 differed from the central haplotype by a single nucleotide and one differed by two nucleotides from the central haplotype. Satellite haplotypes were not shared between the Gulf and Atlantic; five satellite haplotypes were unique to the Gulf and 11 satellite haplotypes were unique to the Atlantic.

Discussion

The primary focus of this study was to test the null hypothesis of genetic homogeneity of *M. canis* in the western Atlantic Ocean (including the northern Gulf of Mexico). Analyses involving nuclear microsatellite loci indicated hierarchal population structure with well-diverged Atlantic and Gulf groups and within ocean basins, while analyses with mtDNA sequences data only indicated significant genetic structuring between the Atlantic and Gulf, likely due in part to low overall haplotype diversity. Genetic structure within ocean basins was not recovered in the Bayesian clustering analysis, but this could be due to the small values of F_{ST}. When F_{ST} values are below .05 (even when significant), STRUCTURE is not capable of detecting differences between populations (Latch et al. 2006).

The mtDNA haplotype diversity (50.3%) and nucleotide diversity (.06%) detected in this study are low compared to other studies that examined population structure of sharks using mtDNA (primarily based on COI; summarized in Karl et al. 2011, Karl et al. 2012, Chabot et al. 2015, Boomer et al. 2012). These low values of diversity are surprising for such an abundant and widespread species, but these patterns are consistent with the low levels of genetic variation seen in other species of *Mustelus* (Boomer et al.

2012; Chabot et al. 2015). The star-contraction seen in the minimum spanning network and high levels of heterozygosity among microsatellite genotypes are indicative that this species may have gone through a recent expansion.

Apparent barriers to gene flow for *M. canis* between the Atlantic and Gulf and within the Gulf are consistent with results seen in other marine taxa. Peninsular Florida in particular has been implicated as a barrier to gene flow for a multitude of other marine taxa (Avise 1992, Gold and Richardson 1998, Gold et al. 2002), likely due to the Loop Current from the Yucatan that exits the Gulf through the Florida Straits (Wiseman and Sturges 1999). The same barrier is seen across a multitude of taxa including invertebrates (Lee et al. 1994, Wicksten and Packard 2005) and several small coastal sharks (*Carcharhinus acronotus*, Portnoy et al. 2014; *C. isodon*, Portnoy et al. 2016). For semitropical/tropical small coastal sharks it has been hypothesized that a combination of strong currents and limited nearshore habitat availability may limit dispersal around peninsular Florida (Portnoy *et al.* 2014).

Within the Gulf, the possibility of eastern and western groups is consistent with what has been seen in *C. acronotus* and *C. isodon*, a pattern hypothesized to result from seasonal migration of both males and females to specific regions for parturition and mating (Portnoy et al. 2014, Portnoy et al. 2016), but further investigation is needed to examine this possibility. For *M. canis,* migration in the Gulf is not well characterized; Female gene flow seems to parallel male gene flow, indicating that female philopatry may not be supported. Estimates of divergence were correlated for bi-parentally inherited microsatellite and maternally inherited mtDNA data.

In summation, the data support two genetically distinct populations of *M. canis*, one in the Atlantic and one in the northern Gulf of Mexico. Further work is warranted to determine whether this is population structure within ocean basins. This work should include tagging and telemetry as well as further molecular assessment of population structure and should employ next-generation sequencing technology, which may increase the power of the analysis.

CHAPTER V

SUMMARY

What Have We learned?

I successfully developed forward and reverse primers to amplify the entire 1047 base-pair NADH-2 gene from species in the genus *Mustelus.* I also developed 15 polymorphic microsatellite loci from an enriched genomic library of *M. canis*. These genetic markers were useful in examining genetic diversity of *M. canis* across its range in U.S. waters and in assessing differences among smoothhound species in the northern Gulf of Mexico. Phylogenetic analysis of 1,047 base pairs of mitochondrially-encoded ND-2 sequences and Bayesian clustering of multi-locus genotypes revealed three genetically distinct and monophyletic lineages (clades) of smoothhound sharks in the northern Gulf of Mexico. Using the molecular markers, I was also able to identify a small number of macroscopically visible characters, which are useful in distinguishing among the smoothhound species in the northern Gulf of Mexico. Though a more systematic study should be performed, I was also able to provide a preliminary assessment of spatial/temporal factors to compare capture localities among species. Given that the smoothhounds in the Gulf can be distinguished with both morphological and molecular markers, accurate assessments of life history can be assessed for these species, two of which are data-deficient and one of which is Near-Threatened according to the International Union for the Conservation of Nature (IUCN 2013).

In the assessment of population structure, I rejected the hypothesis of genetic panmixia across the range of *M. canis* in U.S. waters. Concordant with limited tagging data and observations of seasonal movements, analysis of both mtDNA and microsatellite loci showed that there was little genetic divergence within ocean basins (Atlantic, Gulf), but low levels of genetic divergence were detected between the Gulf and Atlantic, indicating that peninsular Florida may be a barrier to dispersal for this species. Results of genetic analyses suggest that there may have been a recent a recent expansion of *M. canis* across its range in U.S. waters.

Importance of This Study

The results of this study were used as part of the South East Data Assessment and Review (SEDAR) assessment of smoothhound sharks in the U.S Atlantic. The results of this study provide evidence and methods for distinguishing among morphologically similar species of smoothhounds in the northern Gulf of Mexico, which may be useful for studies of basic biology for each of the species. While not within the scope of this study, this work also offers a baseline by which more detailed assessments of life history, demography, and small-scale habitat use and population structure for smoothhound species may be established.

Prior to this study, no information existed regarding genetic diversity/connectivity of smoothhounds in the U.S. Atlantic. The results of this study showed that there were three distinct lineages (species) of smoothhounds in the northern Gulf of Mexico, and

another along the east coast of the United States. While the final SEDAR report acknowledges the distinct lineages, due to the lack of species-specific landing data, confusion in distinguishing among the species in the field, and the conclusion that smoothhounds are not targeted in the northern Gulf of Mexico, the three species (*M. canis, M. norrisi,* and *M. sinusmexicanus)* will be managed as a complex in this region. *Mustelus canis* along the Atlantic coast will be treated as separate stock from those in the Gulf of Mexico (SEDAR 2015).

REFERENCES

Ashe, J.L., Feldheim, K.A., Fields, A.T., Reyier, E.A., Brooks, E.J., O'Connell, M.T., Skomal, G., Gruber, S.H. and Chapman, D.D., 2015. Local population structure and context-dependent isolation by distance in a large coastal shark. *Marine Ecology Progress Series*, *520*, p.203.

Atlantic States Marine Fisheries Commission (ASMFC) 2013. Addendum III to the Interstate Fishery Management Plan for Atlantic Coastal Sharks, 8 pps.

Avise, J. C. 1992. Molecular population structure and the biogeographic history of a regional fauna: a case history with lessons for conservation biology. Oikos, 62-76

Bandelt HJ., Forste, P, and Röhl A. 1999. Median-joining networks for inferring intraspecific phylogenies. Molecular Biology and Evolution, 16(1), 37-48.

Benjamini, Y. and Hochberg, Y., 1995. Controlling the false discovery rate: a practical and powerful approach to multiple testing. Journal of the Royal Statistical Society. Series B (Methodological), pp.289-300.

Bigelow, H. B. and W. C. Schroeder. 1948. Sharks. In J. Tee-Van, C. M. Breder, S. F. Hildebrand, A. E. Parr and W .C. Schroeder (eds.) Fishes of the Western North

Atlantic. Part One. Lancelets, Cyclostomes, Sharks. Sears Foundation for Marine Research, Yale University, New Haven. pp 224-254.

Boomer, J.J. and Stow, A.J., 2010. Rapid isolation of the first set of polymorphic microsatellite loci from the Australian gummy shark, Mustelus antarcticus and their utility across divergent shark taxa. Conservation Genetics Resources, 2(1), pp.393-395.

Boomer, J. J., Harcourt, R. G., Francis, M. P., & Stow, A. J. (2012). Genetic divergence, speciation and biogeography of Mustelus (sharks) in the central Indo-Pacific and Australasia. Molecular Phylogenetics and Evolution, 64(3), 697-703.

Boutin-Ganache, I., Raposo, M., Raymond, M. and Deschepper, C.F., 2001. M13-tailed primers improve the readability and usability of microsatellite analyses performed with two different allele-sizing methods. Biotechniques, 31(1), pp.24-6.

Bretz, F., T. Hothorn, and P. Westfall. 2010. Multiple comparisons using R. Chapman Hall/CRC Press, Boca Raton, FL. 205 pages.

Campagna, S. E., Joyce, W., Marks, L., Hurley, P., Natanson, L. J., Kohler, N. E., ... & Harley, S. (2008). The rise and fall (again) of the porbeagle shark population in the Northwest Atlantic. Sharks of the Open Ocean: Biology, Fisheries and

Conservation, 445-461.

Castro, J. I. 2011. The Sharks of North America. Oxford University Press, USA. 640 pp.

Carlisle, A.B. 2006. Mustelus sinusmexicanus. In: IUCN 2013. IUCN Red List of Threatened Species. Version 2013.2. <www.iucnredlist.org>. Downloaded on 05 April 2014

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 18.3: 545-552.

Chabot, C.L. and Nigenda, S., 2011. Characterization of 13 microsatellite loci for the tope shark, Galeorhinus galeus, discovered with next-generation sequencing and their utility for eastern Pacific smooth-hound sharks (Mustelus). Conservation Genetics Resources, 3(3), pp.553-555.

Chabot, C.L., 2015. Microsatellite loci confirm a lack of population connectivity among globally distributed populations of the tope shark Galeorhinus galeus (Triakidae). Journal of Fish Biology, 87(2), pp.371-385.

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, NJ.

Conrath, C. L. and J. A. Musick. 2002. Reproductive biology of the smooth dogfish, Mustelus canis, in the northwest Atlantic Ocean. Environmental Biology of Fishes 64: 367-377.

Conrath, C. L., Gelsleichter, J., & Musick, J. A. 2002. Age and growth of the smooth dogfish (Mustelus canis) in the northwest Atlantic Ocean. Fishery Bulletin, 100 (4), 674-682.

Conrath, C. 2005. Mustelus canis. The IUCN Red List of Threatened Species 2005: e.T39359A10215463. http://dx.doi.org/10.2305/IUCN.UK.2005.RLTS.T39359A10215463.en. Downloaded on 08 April 2015.

Cortés, E. and H. Balchowsky. 2014. Preliminary catches of smoothhound sharks. SEDAR39- DW-03. SEDAR, North Charleston, SC. 29 pp.

Darriba D, Taboada GL, Doallo R and Posada D. 2012. jModelTest 2: more models, new heuristics and parallel computing. Nature Methods 9(8), 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.

Do, C., Waples, R. S., Peel, D., Macbeth, G. M., Tillett, B. J., & Ovenden, J. R. 2014. NeEstimator v2: re□implementation of software for the estimation of contemporary effective population size (Ne) from genetic data. Molecular Ecology Resources, 14(1), 209-214.

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

Duncan, K.M. and Holland, K.N., 2006. Habitat use, growth rates and dispersal patterns of juvenile scalloped hammerhead sharks Sphyrna lewini in a nursery habitat. Marine Ecology Progress Series, 312, pp.211-221.

Earl, D. A. 2012. STRUCTURE HARVESTER: a website and program for visualizing STRUCTURE output and implementing the Evanno method. Conservation Genetics

Resources, 4(2), 359-361.

Eschmeyer, W. N. (ed). Catalog of Fishes. California Academy of Sciences (http://research.calacademy.org/research/ichthyology/catalog/fishcatmain.asp). Electronic version accessed 10 October 2012.

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.

Excoffier, L., G. Laval, and S. Schneider. 2005. ARLEQUIN (version 3.0): An integrated software package for population genetics data analysis. Evolutionary Bioinformatics Online 1:47-50.

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.

Feldheim, K.A., Gruber, S.H. and Ashley, M.V., 2001. Population genetic structure of the lemon shark (Negaprion brevirostris) in the western Atlantic: DNA microsatellite variation. Molecular Ecology, 10(2), pp.295-303.

Felsenstein, J. 1981. Evolutionary trees from DNA sequences: A maximum likelihood approach. Journal of Molecular Evolution 17:368–376.

Giresi, M., M. A. Renshaw, D. S. Portnoy, and J. R. Gold. 2011. Isolation and characterization of microsatellite markers for the dusky smoothhound shark, Mustelus canis. Conservation Genetics Resources 4: 101-104

Giresi, M.M., Grubbs, D., Portnoy, D.S., Drigger, W., Jones, L., and Gold, J.R. 2015. Identification and distribution of morphologically conserved smoothhound sharks (Genus Mustelus) in the northern Gulf of Mexico. Transactions of the American Fisheries Society 144.6: 1301-1310

Gold JR, Richardson LR. 1998. Population structure in greater amberjack, Seriola dumerili, from the Gulf of Mexico and the western Atlantic Ocean. Fisheries Bulletin, 96, 767-778.

Gold, J. R., Pak, E., & DeVries, D. A. 2002. Population structure of king mackerel (Scomberomorus cavalla) around peninsular Florida, as revealed by microsatellite DNA. Fishery Bulletin, 100(3), 491-509.

Goudet, J. 2005. Hierfstat, a package for R to compute and test hierarchical F□statistics. Molecular Ecology Notes, 5(1), 184-186.

Grubbs, R.D. & Musick, J.A. 2007. Occurrence, catch rates, and length frequencies for smooth dogfish (Mustelus canis) caught in the VIMS Longline Survey: 1974-2006. Report to AMFC Mustelus Working Group, 9 pp.

Guindon, S., & Gascuel, O. 2003.. A simple, fast, and accurate algorithm to estimate large phylogenies by maximum likelihood. Systematic biology, 52(5), 696-704.

Guo, S., and Thompson, E.. 1992. Performing the exact test of Hardy-Weinberg proportion from multiple alleles. Biometrics 48:361-372

Hasegawa M, Kishino H, Yano T (1985). Dating of the human-ape splitting by a molecular clock of mitochondrial DNA. Journal of Molecular Evolution 22 (2): 160–74

Heemstra, P.C. 1973. A Revision of the Shark Genus Mustelus (Squaliformes: Carcharhinidae). University of Miami. P. 187.

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.

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

Karl SA, Castro A.L.F., Lopez J.A., Charvet P., Burgess G.H. 2011. Phylogeography and conservation of the bull sharks (Carcharhinus leucus) inferred from mitochondrial and microsatellite DNA. Conservation Genetics 12: 371-382.

Karl SA, Castro A.L.F., Garla R.C. 2012. Population genetics of the nurse shark (Ginglymostoma cirratum) in the western Atlantic. Marine Biology, 159, 489-498.

Keeney, D. B., M. Heupel, R. E. Hueter, and E. J. Heist. 2005. Microsatellite and mitochondrial DNA analyses of the genetic structure of blacktip shark (Carcharhinus limbatus) nurseries in the Northwestern Atlantic, Gulf of Mexico, and Caribbean Sea. Molecular Ecology 14:1911-1923.

Latch, E.K., Dharmarajan, G., Glaubitx, J.C. and Rhodes, O.E. 2006. Relative perfomances of Bayesian clustering software for inferring population substructure and individual assignment at low levels of population differentiation. Conservation Genetics, 7(2), 295-302

Lê, S., J. Josse, and F. Husson. 2008. FactoMineR: an R Package for multivariate analysis. Journal of Statistical Software 25: 1-18.

Lee, T.N., Clarke, M.E., Williams, E., Szmant, A., Berger, T., 1994. Evolution of the Tortugas Gyre and its influence on recruitment in the Florida Keys. Bulletin of Marine Science 54: 621–646.

Lewis, P.O. and Zaykin, D., 2002. GDA (Genetic Data Analysis). Software distributed by the authors.

Lopez, J.A., Ryburn J.A., Fedrigo, O., Naylor G.J.P. 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

Meirmans, P.G., and P.H. Van Tienderen: (2004), GENOTYPE and GENODIVE: two programs for the analysis of genetic diversity of asexual organisms, Molecular Ecology Notes 4 p.792-794.

Miller, M.A., Pfeiffer, W., and Schwartz, T. 2010. “Creating the CIPRES Science Gateway for inference of large phylogenetic trees" in Proceedings of the Gateway Computing Environments Workshop (GCE), 14 Nov. 2010, New Orleans, LA pp1-8.

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., Burgess, G., Cailliet, G., Camhi, M., & Fordham, S. (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.

Nei, M., 1987. Molecular evolutionary genetics. Columbia university press.

NMFS 2010a. Final Amendment 3 to the Consolidated Atlantic Highly Migratory Species Fishery Management Plan. Highly Migratory Species Management Division, 1315 East West Highway, Silver Spring, MD.

NMFS 2010b. Guide for Complying with the Atlantic Shark Fisheries Regulations in Amendment 3 to the Consolidated HMS FMP. Highly Migratory Species Management Division, 1315 East West Highway, Silver Spring, MD.

Plank, S.M., Lowe, C.G., Feldheim, K.A., Wilson, R.R. and Brusslan, J.A., 2010. Population genetic structure of the round stingray Urobatis halleri (Elasmobranchii: Rajiformes) in southern California and the Gulf of California.Journal of Fish

Biology, 77(2), pp.329-340.

Portnoy D. S., J. R. McDowell, C. T. McCandless, J. A. Musick and J. E. Graves. 2009. Effective size closely approximates the census size in the heavily exploited western Atlantic population of sandbar sharks, Carcharhinus plumbeus. Conservation Genetics 10: 1697-1705

Portnoy, D. S., Hollenbeck, C. M., Belcher, C. N., Driggers, W. B., Frazier, B. S., Gelsleichter, J., & Gold, J. R. 2014. Contemporary population structure and post□ glacial genetic demography in a migratory marine species, the blacknose shark, Carcharhinus acronotus. Molecular Ecology, 23(22), 5480-5495.

Portnoy, D.S., Hollenbeck, C.M, Bethea, D.M., Frazier, B.S., Gelsleicther, J., Gold, J.R. 2016. Population structure, gene flow, and historical demography of a small coastal shark (Carcharhinus isodon) in U.S. waters of the western Atlantic Ocean. ICES Journal of Marine Science. Submitted.

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, Austria.

http://www.R-project.org.

Rambaut, A. (2009). FigTree version 1.3. 1. Computer program distributed by the author, website: http://tree. bio. ed. ac. uk/software/figtree/.

Raymond, M., and F. Rousset. 1995. GENEPOP (version 1.2): Population genetics software for exact tests and ecumenicism. Heredity 86:248-249.

Renshaw, M.A., Portnoy, D.S. and Gold, J.R., 2010. PCR primers for nuclear-encoded microsatellites of the groupers Cephalopholis fulva (coney) and Epinephelus guttatus (red hind). Conservation Genetics, 11(3), pp.1197-1202.

Rice, W. R. 1989. Analyzing tables of statistical tests. Evolution 43:223-225.

Rousset, F., 2008. genepop'007: a complete re□implementation of the genepop software for Windows and Linux. Molecular Ecology Resources, 8(1), pp.103-106.

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

Schneider, S., D. Roessli, and L. Excoffier. 2000. ARLEQUIN version 2.00: a software

for population genetic data analysis. In Genetics and Biometry Laboratory, Department of Anthropology, University of Geneva, Geneva Switzerland.

SEDAR. 2014. Southeast Data, Assessment, and Review: SEDAR 39 HMS Atlantic Smoothhound Shark Section: Data Workshop Report. Available at <http://www.sefsc.noaa.gov/sedar/download/S39_Atl_canis_DW_Report_final_w_disclaimer_watermark.pdf?id=DOCUMENT, 62 pp.

SEDAR. 2015. Southeast Data, Assessment, and review: SEDAR 39 HMS Gulf of Mexico Smoothhound Complex: Section III: Assessment Process Report. Available at <http://www.sefsc.noaa.gov/sedar/download/S39_Smoothhound%20complex_AW_Report_Final%20for_RP_WATERMARK.pdf?id=DOCUMENT>, 227 pp.

Jones, L. Driggers W., Hannan, K., Hoffmayer E., Jones, C. 2014. Identification, Life History and Distribution of Mustelus canis, M. norrisi and M. sinusmexicanus in the northern Gulf of Mexico. SEDAR39-DW-22. SEDAR. North Charleston, SC. 26 pp.

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.

Skomal GB. Shark Nursery Areas in the Coastal Waters of Massachusetts. 2007. American Fisheries Society Symposium 50:17-33.

Smouse, P. E., Long, J. C., and Sokal, R. R. 1986. Multiple regression and correlation extensions of the Mantel test of matrix correspondence. Systematic Zoology: 627-632.

Springer, S., and Lowe, RH. 1963. A new smooth dogshark, Mustelus higmani, from the equatorial Atlantic Coast of South America. Copeia: 1963: 245-251.

Stevens, J. D., Bonfil, R., Dulvy, N. K., & Walker, P. A. (2000). The effects of fishing on sharks, rays, and chimaeras (chondrichthyans), and the implications for marine ecosystems. ICES Journal of Marine Science: Journal du Conseil, 57(3), 476-494.

Sukumaran, J. and Mark T. Holder. 2010. DendroPy: A Python library for phylogenetic computing. Bioinformatics 26: 1569-1571.

Tamura, K., Stecher, G., Peterson, D., Filipski, A. and Kumar, S., 2013. MEGA6: molecular evolutionary genetics analysis version 6.0. Molecular biology and evolution, p.mst197.

TeWinkle, L. E. 1950. Notes on ovulation, ova and early development in the smooth dogfish, Mustelus canis. Biological Bulletin 99: 474486.

van Oosterhout, C., W. F. Hutchinson, D. P. M. Wills, and P. Shipley. 2004. MICRO-CHECKER: software for identifying genotyping errors in microsatellite data. Molecular Ecology Notes 4:535-538.

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. CSIRO, Hobart.

Wicksten, M.K. and Packard, J.M., 2005. A qualitative zoogeographic analysis of decapod crustaceans of the continental slopes and abyssal plain of the Gulf of Mexico. Deep Sea Research Part I: Oceanographic Research Papers, 52(9), pp.1745-1765.

Wiseman, W.J. and Sturges, W., 1999. Physical oceanography of the Gulf of Mexico: Processes that regulate its biology. The Gulf of Mexico Large Marine Ecosystem: assessment, sustainability and management, H. Kumpf, K. Steidinger and K. Sherman (eds.). Blackwell Science, New York, pp.77-92.

Zwickl, D. J. 2006. Genetic algorithm approaches for the phylogenetic analysis of large biological sequence datasets under the maximum likelihood criterion. Ph.D. dissertation, The University of Texas at Austin, 125 pp.

APPENDIX I

MORPHOLOGICAL KEY TO DISTINGUISH AMONG 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 biserial, extending to the first gill slit; nasal flaps narrow with a concave or angular posterior margin . . . .*M. sinusmexicanus*

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 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 . . . . . *M. canis*

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 . . . . . *M. norrisi*

Table A 4.1 Sample data for all *Mustelus canis* individuals included in the population structure analyses. The sampling outfit, and capture coordinates for each individual are listed.

Sample_Name	Sampling_Outfit	Latitude	Longitude
DB_001	Apex Predators Program	39.02005	-75.06718
DB_002	Apex Predators Program	39.02005	-75.06718
DB_003	Apex Predators Program	39.02005	-75.06718
DB_004	Apex Predators Program	39.02005	-75.06718
DB_005	Apex Predators Program	39.02005	-75.06718
DB_006	Apex Predators Program	39.02005	-75.06718
DB_007	Apex Predators Program	39.02005	-75.06718
DB_008	Apex Predators Program	39.05278	-75.04480
DB_009	Apex Predators Program	39.05278	-75.04480
DB_010	Apex Predators Program	39.05278	-75.04480

Table A 4.1 Continued (2)

Sample_Name	Sampling_Outfit	Latitude	Longitude
DB_011	Apex Predators Program	39.18689	-75.05150
DB_012	Apex Predators Program	39.18689	-75.05150
DB_013	Apex Predators Program	39.12395	-74.90903
DB_014	Apex Predators Program	38.94618	-75.08132
DB_015	Apex Predators Program	38.94618	-75.08132
DB_016	Apex Predators Program	38.97047	-75.08358
DB_017	Apex Predators Program	38.97047	-75.08358
DB_018	Apex Predators Program	39.08175	-75.01739
DB_019	Apex Predators Program	39.03835	-74.96890
DB_020	Apex Predators Program	39.03835	-74.96890
DB_021	Apex Predators Program	38.98740	-75.04102
DB_022	Apex Predators Program	38.98740	-75.04102
DB_023	Apex Predators Program		
DB_024	Apex Predators Program	38.85717	-75.08161
DB_025	Apex Predators Program	38.85717	-75.08161
DB_026	Apex Predators Program	38.85717	-75.08161
DB_027	Apex Predators Program	38.85717	-75.08161
DB_028	Apex Predators Program	38.85717	-75.08161
DB_029	Apex Predators Program	38.85717	-75.08161
DB_030	Apex Predators Program	38.85717	-75.08161
DB_031	Apex Predators Program	38.85717	-75.08161
DB_032	Apex Predators Program	38.85717	-75.08161
DB_033	Apex Predators Program	38.85717	-75.08161
DB_034	Apex Predators Program	38.85717	-75.08161
DB_035	Apex Predators Program	39.08216	-75.24796
DB_036	Apex Predators Program	39.01198	-75.24162
DB_038	Apex Predators Program	38.99688	-75.27960
DB_039	Apex Predators Program	38.99688	-75.27960
DB_040	Apex Predators Program	38.94951	-75.17765
DB_041	Apex Predators Program	38.94951	-75.17765
DB_042	Apex Predators Program	38.94951	-75.17765
DB_043	Apex Predators Program	38.94457	-75.17144
DB_044	Apex Predators Program	38.91602	-75.21479
DB_045	Apex Predators Program	38.85053	-75.21173
DB_046	Apex Predators Program	38.96563	-75.08168
DB_047	Apex Predators Program	38.96563	-75.08168
DB_048	Apex Predators Program	38.96563	-75.08168

Table A 4.1 Continued (3)

Sample_Name	Sampling_Outfit	Latitude	Longitude
DB_049	Apex Predators Program	39.02643	-75.06513
DB_050	Apex Predators Program	39.05069	-75.02147
DB_051	Apex Predators Program	39.06462	-75.20215
DB_052	Apex Predators Program	39.06462	-75.20215
DB_053	Apex Predators Program	39.06462	-75.20215
DB_054	Apex Predators Program	39.06462	-75.20215
DB_055	Apex Predators Program	39.06462	-75.20215
DB_056	Apex Predators Program	39.06462	-75.20215
DB_057	Apex Predators Program	39.05912	-75.13030
DB_058	Apex Predators Program	39.12203	-75.30017
DB_059	Apex Predators Program	39.13890	-75.39438
DB_060	Apex Predators Program	39.16582	-75.28568
DB_061	Apex Predators Program	39.16582	-75.28568
DB_062	Apex Predators Program	39.16582	-75.28568
DB_063	Apex Predators Program	39.16582	-75.28568
DB_064	Apex Predators Program	39.16582	-75.28568
DB_065	Apex Predators Program	39.16582	-75.28568
DB_066	Apex Predators Program	39.16582	-75.28568
DB_067	Apex Predators Program	39.16582	-75.28568
DB_068	Apex Predators Program	39.16582	-75.28568
DB_069	Apex Predators Program	39.12510	-75.18402
DB_070	Apex Predators Program	39.12510	-75.18402
DB_071	Apex Predators Program	39.12510	-75.18402
DB_072	Apex Predators Program	39.14788	-75.15188
DB_073	Apex Predators Program	39.14788	-75.15188
DB_074	Apex Predators Program	39.14788	-75.15188
DB_075	Apex Predators Program	39.17948	-75.08350
DB_076	Apex Predators Program	39.17948	-75.08350
DB_077	Apex Predators Program	39.16188	-75.03458
DB_078	Apex Predators Program	39.16188	-75.03458
DB_079	Apex Predators Program	39.16188	-75.03458
DB_080	Apex Predators Program	39.05245	-75.23873
DB_081	Apex Predators Program	39.05245	-75.23873
DB_082	Apex Predators Program	39.05245	-75.23873
DB_083	Apex Predators Program	39.05245	-75.23873
DB_084	Apex Predators Program	39.05245	-75.23873
DB_085	Apex Predators Program	39.05245	-75.23873

Table A 4.1 Continued (4)

Sample_Name	Sampling_Outfit	Latitude	Longitude
DB_086	Apex Predators Program	39.05245	-75.23873
DB_087	Apex Predators Program	39.05642	-75.37680
DB_088	Apex Predators Program	39.05642	-75.37680
DB_089	Apex Predators Program	39.01497	-75.28618
DB_090	Apex Predators Program	39.01497	-75.28618
DB_091	Apex Predators Program	39.01497	-75.28618
DB_092	Apex Predators Program	39.08770	-75.01583
DB_093	Apex Predators Program	38.96358	-75.08115
DB_094	Apex Predators Program	38.96358	-75.08115
DB_095	Apex Predators Program	38.96358	-75.08115
DB_096	Apex Predators Program	38.96358	-75.08115
DB_097	Apex Predators Program	38.96358	-75.08115
DB_098	Apex Predators Program	38.96358	-75.08115
DB_099	Apex Predators Program	38.96358	-75.08115
DB_100	Apex Predators Program	38.96358	-75.08115
DB_101	Apex Predators Program	38.96358	-75.08115
DB_102	Apex Predators Program	38.96358	-75.08115
DB_103	Apex Predators Program	38.96358	-75.08115
DB_104	Apex Predators Program	38.96358	-75.08115
DB_105	Apex Predators Program	38.96358	-75.08115
DB_106	Apex Predators Program	38.96358	-75.08115
DB_107	Apex Predators Program	38.85592	-75.08722
DB_108	Apex Predators Program	38.85592	-75.08722
DB_109	Apex Predators Program	38.85592	-75.08722
DB_110	Apex Predators Program	38.85592	-75.08722
DB_111	Apex Predators Program	38.85592	-75.08722
DB_112	Apex Predators Program	39.06035	-75.26662
DB_113	Apex Predators Program	39.06035	-75.26662
DB_114	Apex Predators Program	39.06035	-75.26662
DB_115	Apex Predators Program	39.06035	-75.26662
DB_116	Apex Predators Program	39.06035	-75.26662
DB_117	Apex Predators Program	39.06035	-75.26662
DB_118	Apex Predators Program	39.06035	-75.26662
DB_119	Apex Predators Program	38.95037	-75.17787
DB_120	Apex Predators Program	38.95037	-75.17787
DB_121	Apex Predators Program	38.95037	-75.17787
DB_122	Apex Predators Program	38.95037	-75.17787

Table A 4.1 Continued (5)

Sample_Name	Sampling_Outfit	Latitude	Longitude
DB_123	Apex Predators Program	38.95037	-75.17787
DB_124	Apex Predators Program	38.95037	-75.17787
DB_125	Apex Predators Program	38.95037	-75.17787
DB_126	Apex Predators Program	38.99237	-75.22467
DB_127	Apex Predators Program	38.99237	-75.22467
DB_128	Apex Predators Program	38.99237	-75.22467
DB_129	Apex Predators Program	38.94243	-75.08208
DB_130	Apex Predators Program	38.94243	-75.08208
DB_131	Apex Predators Program	38.94243	-75.08208
DB_132	Apex Predators Program	38.94243	-75.08208
DB_133	Apex Predators Program	38.94243	-75.08208
DB_134	Apex Predators Program	38.94243	-75.08208
DB_135	Apex Predators Program	38.96718	-75.08400
DB_136	Apex Predators Program	38.96718	-75.08400
DB_137	Apex Predators Program	38.96718	-75.08400
DB_138	Apex Predators Program	39.01492	-75.06108
DB_139	Apex Predators Program	39.01492	-75.06108
DB_140	Apex Predators Program	39.05618	-75.04253
DB_141	Apex Predators Program	39.05618	-75.04253
MA_001	MA Dept of Marine Fisheries		
MA_002	MA Dept of Marine Fisheries		
MA_003	MA Dept of Marine Fisheries	41.297251	-71.030984
MA_004	MA Dept of Marine Fisheries	41.185286	-70.333781
MA_005	MA Dept of Marine Fisheries	41.233944	-71.018322
MA_006	MA Dept of Marine Fisheries	41.233944	-71.018322
MA_007	MA Dept of Marine Fisheries	41.211525	-70.565529
MA_008	MA Dept of Marine Fisheries	41.211525	-70.565529
MA_009	MA Dept of Marine Fisheries		
MA_010	MA Dept of Marine Fisheries	41.2793	-70.285
MA_011	MA Dept of Marine Fisheries	41.2793	-70.285
MA_012	MA Dept of Marine Fisheries	41.2957	-70.2976
MA_013	MA Dept of Marine Fisheries	41.2957	-70.2976
MA_014	MA Dept of Marine Fisheries	41.2957	-70.2976
MA_015	MA Dept of Marine Fisheries	41.2957	-70.2976
MA_016	MA Dept of Marine Fisheries	41.2957	-70.2976
MA_017	MA Dept of Marine Fisheries	41.2957	-70.2976
MA_018	MA Dept of Marine Fisheries	41.2957	-70.2976

Table A 4.1 Continued (6)

Sample_Name	Sampling_Outfit	Latitude	Longitude
MA_019	MA Dept of Marine Fisheries	41.2957	-70.2976
MA_020	MA Dept of Marine Fisheries	41.2957	-70.2976
MA_021	MA Dept of Marine Fisheries	41.2957	-70.2976
MA_022	MA Dept of Marine Fisheries	41.2957	-70.2976
MA_023	MA Dept of Marine Fisheries	41.2957	-70.2976
MA_024	MA Dept of Marine Fisheries	41.2957	-70.2976
MA_025	MA Dept of Marine Fisheries	41.2957	-70.2976
MA_026	MA Dept of Marine Fisheries	41.3229	-70.2758
MA_027	MA Dept of Marine Fisheries	41.3229	-70.2758
MA_028	MA Dept of Marine Fisheries	41.3229	-70.2758
MA_029	MA Dept of Marine Fisheries	41.3229	-70.2758
MA_030	MA Dept of Marine Fisheries	41.3229	-70.2758
MA_031	MA Dept of Marine Fisheries	41.3229	-70.2758
MA_032	MA Dept of Marine Fisheries	41.3318	-70.1952
MA_033	MA Dept of Marine Fisheries	41.3318	-70.1952
MA_034	MA Dept of Marine Fisheries	41.3318	-70.1952
MA_035	MA Dept of Marine Fisheries	41.3318	-70.1952
MA_036	MA Dept of Marine Fisheries	41.3318	-70.1952
MA_037	MA Dept of Marine Fisheries	41.3318	-70.1952
MA_038	MA Dept of Marine Fisheries	41.3318	-70.1952
MA_039	MA Dept of Marine Fisheries	41.3318	-70.1952
MA_040	MA Dept of Marine Fisheries	41.3318	-70.1952
MA_041	MA Dept of Marine Fisheries	41.3318	-70.1952
MA_042	MA Dept of Marine Fisheries	41.3318	-70.1952
MA_043	MA Dept of Marine Fisheries	41.3318	-70.1952
MA_044	MA Dept of Marine Fisheries	41.3318	-70.1952
MA_045	MA Dept of Marine Fisheries	41.3318	-70.1952
MA_046	MA Dept of Marine Fisheries	41.3318	-70.1952
MA_047	MA Dept of Marine Fisheries	41.3318	-70.1952
MA_048	MA Dept of Marine Fisheries	41.3318	-70.1952
MA_049	MA Dept of Marine Fisheries	41.3318	-70.1952
MA_050	MA Dept of Marine Fisheries	41.3318	-70.1952
MA_051	MA Dept of Marine Fisheries	41.3318	-70.1952
MA_052	MA Dept of Marine Fisheries	41.3318	-70.1952
MA_053	MA Dept of Marine Fisheries	41.3318	-70.1952
MA_054	MA Dept of Marine Fisheries	41.3318	-70.1952
MA_055	MA Dept of Marine Fisheries	41.3318	-70.1952

Table A 4.1 Continued (7)

Sample_Name	Sampling_Outfit	Latitude	Longitude
MA_056	MA Dept of Marine Fisheries	41.3318	-70.1952
MA_057	MA Dept of Marine Fisheries	41.3318	-70.1952
MA_058	MA Dept of Marine Fisheries	41.3318	-70.1952
MA_059	MA Dept of Marine Fisheries	41.4003	-70.4441
MA_060	MA Dept of Marine Fisheries	41.4003	-70.4441
MA_061	MA Dept of Marine Fisheries		
MA_079	MA Dept of Marine Fisheries	41.3258	-70.4895
MA_080	MA Dept of Marine Fisheries	41.3258	-70.4895
MA_081	MA Dept of Marine Fisheries	41.3258	-70.4895
MA_082	MA Dept of Marine Fisheries	41.3258	-70.4895
MA_083	MA Dept of Marine Fisheries	41.3258	-70.4895
MA_084	MA Dept of Marine Fisheries	41.3258	-70.4895
MA_085	MA Dept of Marine Fisheries	41.3258	-70.4895
MA_086	MA Dept of Marine Fisheries	41.3258	-70.4895
MA_087	MA Dept of Marine Fisheries	41.3258	-70.4895
MA_088	MA Dept of Marine Fisheries	41.3752	-70.0497
MA_089	MA Dept of Marine Fisheries	41.3752	-70.0497
MA_090	MA Dept of Marine Fisheries	41.3752	-70.0497
MA_091	MA Dept of Marine Fisheries	41.3752	-70.0497
MA_092	MA Dept of Marine Fisheries	41.3640	-70.0792
MA_093	MA Dept of Marine Fisheries	41.3640	-70.0792
MA_094	MA Dept of Marine Fisheries	41.3373	-70.1393
MA_095	MA Dept of Marine Fisheries	41.3373	-70.1393
MA_101	MA Dept of Marine Fisheries	41.4587	-70.0659
MA_102	MA Dept of Marine Fisheries	41.5707	-70.2165
MA_103	MA Dept of Marine Fisheries	41.8155	-70.0916
MA_104	MA Dept of Marine Fisheries	41.5448	-70.1907
MA_105	MA Dept of Marine Fisheries	41.5448	-70.1907
MA_115	MA Dept of Marine Fisheries	41.5225	-70.1304
MA_120	MA Dept of Marine Fisheries	41.5225	-70.1304
MA_127	MA Dept of Marine Fisheries	41.5323	-70.2979
MA_140	MA Dept of Marine Fisheries	41.5323	-70.2979
MA_149	MA Dept of Marine Fisheries	41.4240	-70.1388
MA_150	MA Dept of Marine Fisheries	41.4240	-70.1388
MA_151	MA Dept of Marine Fisheries	41.3672	-70.0723
MA_152	MA Dept of Marine Fisheries	41.3672	-70.0723
MA_153	MA Dept of Marine Fisheries	41.3672	-70.0723

Table A 4.1 Continued (8)

Sample_Name	Sampling_Outfit	Latitude	Longitude
MA_154	MA Dept of Marine Fisheries	41.3672	-70.0723
MA_155	MA Dept of Marine Fisheries	41.4287	-70.3570
MA_156	MA Dept of Marine Fisheries	41.4287	-70.3570
MA_157	MA Dept of Marine Fisheries	41.4287	-70.3570
MA_158	MA Dept of Marine Fisheries	41.4760	-70.4178
MA_159	MA Dept of Marine Fisheries	41.4760	-70.4178
MA_161	MA Dept of Marine Fisheries	41.3672	-70.0723
MA_162	MA Dept of Marine Fisheries	41.3672	-70.0723
MA_163	MA Dept of Marine Fisheries	41.5455	-70.7111
MA_164	MA Dept of Marine Fisheries	41.3672	-70.0723
MA_165	MA Dept of Marine Fisheries	41.5455	-70.7111
MA_173	MA Dept of Marine Fisheries	41.6497	-70.7467
MA_175	MA Dept of Marine Fisheries	41.5924	-70.8073
MA_191	MA Dept of Marine Fisheries	41.4390	-70.2716
MA_192	MA Dept of Marine Fisheries	41.4390	-70.2716
MA_198	MA Dept of Marine Fisheries	41.5029	-70.3967
MA_199	MA Dept of Marine Fisheries	41.5029	-70.3967
MA_200	MA Dept of Marine Fisheries	41.5029	-70.3967
MaCa	MA Dept of Marine Fisheries		
Mcan_FL002	Florida State University	29.14570	-86.27903
Mcan_FL003	Florida State University	29.07300	-88.61877
Mcan_FL004	Florida State University	29.07300	-88.61877
Mcan_FL005	Florida State University	26.8062	-84.73701
Mcan_FL006	Florida State University	29.43328	-87.29511
Mcan_FL007	Florida State University	29.06965	-88.63912
Mcan_FL008	Florida State University	29.4084	-87.3594
Mcan_FL009	Florida State University	29.3013	-87.7754
Mcan_FL010	Florida State University	29.30737	-86.49824
Mcan_FL011	Florida State University	29.4084	-87.3594
Mcan_FL012	Florida State University	29.51875	-86.79906
Mcan_FL013	Florida State University	29.11805	-86.13382
Mcan_FL014	Florida State University	29.14394	-86.28355
Mcan_FL015	Florida State University	29.30737	-86.49824
Mcan_FL016	Florida State University	29.2971	-87.7848
Mcan_FL017	Florida State University	29.51875	-86.79906
Mcan_FL018	Florida State University	29.47424	-87.38697
Mcan_FL019	Florida State University	29.47424	-87.38697

Table A 4.1 Continued (9)

Sample_Name	Sampling_Outfit	Latitude	Longitude
Mcan_FL020	Florida State University	29.11805	-86.13382
Mcan_FL021	Florida State University	29.14394	-86.28355
Mcan_FL022	Florida State University	29.4084	-87.3594
Mcan_FL023	Florida State University	29.2971	-87.7848
Mcan_FL024	Florida State University	29.30737	-86.49824
Mcan_FL025	Florida State University	29.11805	-86.13382
Mcan_FL026	Florida State University	29.30737	-86.49824
Mcan_FL027	Florida State University	29.40836	-87.35937
Mcan_FL028	Florida State University	29.47424	-87.38697
Mcan_FL029	Florida State University	29.30357	-86.33672
Mcan_FL030	Florida State University	29.51875	-86.79906
Mcan_FL031	Florida State University	29.51875	-86.79906
Mcan_FL032	Florida State University	29.40836	-87.35937
Mcan_FL033	Florida State University	29.51875	-86.79906
Mcan_FL034	Florida State University	29.14394	-86.28355
Mcan_FL035	Florida State University	29.51875	-86.79906
Mcan_MS001	NOAA/NMFS Pascagoula	27.2365	-96.3090
Mcan_MS004	NOAA/NMFS Pascagoula	28.2832	-85.4798
Mcan_MS005	NOAA/NMFS Pascagoula	29.5228	-87.3928
Mcan_MS007	NOAA/NMFS Pascagoula	27.5587	-94.6213
Mcan_MS009	NOAA/NMFS Pascagoula	28.0500	-90.7225
Mcan_MS010	NOAA/NMFS Pascagoula	28.8170	-89.3102
Mcan_MS011	NOAA/NMFS Pascagoula	29.5228	-87.3928
Mcan_MS012	NOAA/NMFS Pascagoula	25.2983	-84.3447
Mcan_MS014	NOAA/NMFS Pascagoula	28.0335	-90.5147
Mcan_MS016	NOAA/NMFS Pascagoula	29.3367	-87.7737
Mcan_MS017	NOAA/NMFS Pascagoula	28.0472	-90.6633
Mcan_MS018	NOAA/NMFS Pascagoula	29.3075	-85.9762
Mcan_MS019	NOAA/NMFS Pascagoula	26.3127	-84.5852
Mcan_MS023	NOAA/NMFS Pascagoula	27.6953	-95.6492
Mcan_MS024	NOAA/NMFS Pascagoula	28.7957	-85.1162
MS_MS054	NOAA/NMFS Pascagoula	28.8933	-85.3688
MS_MS055	NOAA/NMFS Pascagoula	29.3217	-87.8482
MS_MS056	NOAA/NMFS Pascagoula	29.3217	-87.8482
MS_MS057	NOAA/NMFS Pascagoula	29.4225	-87.8613
MS_MS059	NOAA/NMFS Pascagoula	27.9413	-91.3607
MS_MS064	NOAA/NMFS Pascagoula	28.2042	-90.3862

Table A 4.1 Continued (10)

Sample_Name	Sampling_Outfit	Latitude	Longitude
MS_MS066	NOAA/NMFS Pascagoula	26.6603	-96.3503
MS_MS068	NOAA/NMFS Pascagoula	28.9470	-85.5422
MS_MS069	NOAA/NMFS Pascagoula	27.5068	-96.0350
MS_MS070	NOAA/NMFS Pascagoula	25.8695	-84.3187
MS_MS073	NOAA/NMFS Pascagoula	27.3513	-84.4037
MS_MS074	NOAA/NMFS Pascagoula	26.8752	-96.4357
MS_MS078	NOAA/NMFS Pascagoula	27.3513	-84.4037
MS_MS081	NOAA/NMFS Pascagoula	26.8620	-96.4002
MS_MS082	NOAA/NMFS Pascagoula	29.4225	-87.8613
MS_MS086	NOAA/NMFS Pascagoula	29.3217	-87.8482
MS_MS089	NOAA/NMFS Pascagoula	28.0550	-84.9582
MS_MS090	NOAA/NMFS Pascagoula	27.6682	-93.4127
MS_MS091	NOAA/NMFS Pascagoula	26.7772	-84.5522
MS_MS097	NOAA/NMFS Pascagoula	29.3408	-87.8565
MS_MS098	NOAA/NMFS Pascagoula	27.8507	-91.7718
MS_MS099	NOAA/NMFS Pascagoula	29.5352	-86.7335
MS_MS102	NOAA/NMFS Pascagoula	28.8933	-85.3688
MS_MS103	NOAA/NMFS Pascagoula	28.8933	-85.3688
MS_MS104	NOAA/NMFS Pascagoula	29.4225	-87.8613
MS_MS105	NOAA/NMFS Pascagoula	27.5068	-96.0350
MS_MS106	NOAA/NMFS Pascagoula	29.3792	-87.9340
MS_MS107	NOAA/NMFS Pascagoula	29.9360	-86.4645
MS_MS111	NOAA/NMFS Pascagoula	27.3513	-84.4037
MS_MS112	NOAA/NMFS Pascagoula	28.0057	-84.6227
MS_MS113	NOAA/NMFS Pascagoula	29.0788	-88.9607
MS_MS114	NOAA/NMFS Pascagoula	28.5790	-89.4502
MS_MS116	NOAA/NMFS Pascagoula	28.8933	-85.3688
MS_MS118	NOAA/NMFS Pascagoula	29.8573	-87.2703
MS_MS119	NOAA/NMFS Pascagoula	27.3513	-84.4037
MS_MS121	NOAA/NMFS Pascagoula	29.3217	-87.8482
MS_MS143	NOAA/NMFS Pascagoula	26.8210	-96.4507
MS_MS144	NOAA/NMFS Pascagoula	26.8210	-96.4507
MS_MS154	NOAA/NMFS Pascagoula	27.5610	-96.0450
MS_MS170	NOAA/NMFS Pascagoula	29.1255	-88.7513
MS_MS171	NOAA/NMFS Pascagoula	29.1255	-88.7513
SC_007	SC Dept of Natural Resources	34.6878	-76.8084
SC_009	SC Dept of Natural Resources	34.6878	-76.8084

Table A 4.1 Continued (11)

Sample_Name	Sampling_Outfit	Latitude	Longitude
SC_010	SC Dept of Natural Resources	34.6878	-76.8084
SC_011	SC Dept of Natural Resources	34.66176667	-77.0026
SC_012	SC Dept of Natural Resources	34.6878	-76.8084
SC_013	SC Dept of Natural Resources	34.6878	-76.8084
SC_014	SC Dept of Natural Resources	34.6878	-76.8084
SC_015	SC Dept of Natural Resources	34.6878	-76.8084
SC_016	SC Dept of Natural Resources	34.6878	-76.8084
SC_017	SC Dept of Natural Resources	34.6667	-76.6335
SC_018	SC Dept of Natural Resources	34.6667	-76.6335
SC_019	SC Dept of Natural Resources	34.6667	-76.6335
SC_020	SC Dept of Natural Resources	35.22681667	-75.5951
SC_021	SC Dept of Natural Resources	35.22681667	-75.5951
SC_022	SC Dept of Natural Resources	35.19468333	-75.7049
SC_023	SC Dept of Natural Resources	35.19468333	-75.7049
SC_024	SC Dept of Natural Resources	34.65523333	-77.0356
SC_025	SC Dept of Natural Resources	33.85498333	-78.0719
SC_026	SC Dept of Natural Resources	35.16771667	-75.79175
SC_027	SC Dept of Natural Resources	35.0336	-76.0322
SC_029	SC Dept of Natural Resources	35.10105	-75.9466
SC_030	SC Dept of Natural Resources	34.65523333	-77.0356
SC_031	SC Dept of Natural Resources	34.62271667	-77.1335
SC_077	SC Dept of Natural Resources	33 00.57	-79 29.12
SC_078	SC Dept of Natural Resources	33 00.57	-79 29.12
SC_079	SC Dept of Natural Resources	33 00.57	-79 29.12
SC_080	SC Dept of Natural Resources	33 00.57	-79 29.12
SC_081	SC Dept of Natural Resources	33 00.57	-79 29.12
SC_082	SC Dept of Natural Resources	33 00.57	-79 29.12
SC_083	SC Dept of Natural Resources	33.817	-79.99
SC_084	SC Dept of Natural Resources	33.817	-79.99
SC_085	SC Dept of Natural Resources	34.652	-77.048
SC_086	SC Dept of Natural Resources	33.817	-79.99
SC_087	SC Dept of Natural Resources	33 01.99	-79 31.76
SC_088	SC Dept of Natural Resources	34.652	-77.048
SC_090	SC Dept of Natural Resources	33 00.57	-79 29.12
SC_091	SC Dept of Natural Resources	33 00.57	-79 29.12

Table A 4.2 Summary statistics of nuclear encoded microsatellite loci for localities of *Mustelus canis* in the Atlantic Ocean and northern Gulf of Mexico. MA, Massachusetts; DB, Delaware Bay; SC, South Carolina; FL and MS east Gulf; TX west Gulf. Summary statistics are also reported for the Atlantic (inclusive of MA, DB, SC) and Gulf (FL, MS, TX). The following statistics are reported: sample size (*n)*, number of allele (#A), rarified allelic richness (A_R), expected heterozygosity (H_E), observed heterozygosity (H_O), probability of conforming to Hardy-Weinberg expectations (P_{HW}), and the inbreeding coefficient (F_{IS})

Microsatellite	MA	DB	SC	Egulf	wGulf	Atlantic	Gulf
Mca31							
n^a	111	140	37	69	20	288	89
#A[b]	5	5	3	3	2	6	4
A_R[c]	3.248	3.405	2.915	2.801	1.999	8.433	7.342
H_E[d]	0.465	0.520	0.381	0.217	0.361	0.482	0.249
H_O[e]	0.342	0.357	0.216	0.245	0.150	0.326	0.146
P_{HW}[f]	0.300	0.292	0.077	0.222	0.337	0.064	0.181
F_{IS}[g]	0.303	0.313	0.433	0.333	0.584	0.322	0.414
Mca33							
N	111	140	37	69	20	288	89
#A	2	2	2	2	1	2	2
A_R	1.283	1.323	1.459	1.362	1.000	4.460	4.607
H_E	0.036	0.042	0.054	0.057	0.000	0.041	0.045
H_O	0.018	0.021	0.027	0.029	0.000	0.021	0.023
P_{HW}	0.996	0.996	1.000	1.000	---	0.009	-0.006
F_{IS}[g]	0.497	0.495	0.500	0.494	NA	0.493	0.496
Mca44							
N	111	140	37	69	20	288	89
#A	8	11	7	7	6	11	7
A_R	6.254	6.456	6.286	6.193	5.921	13.060	11.104
H_E	0.857	0.832	0.847	0.814	0.847	0.843	0.820
H_O	0.874	0.871	0.919	0.841	0.800	0.879	0.832
P_{HW}	0.178	0.503	0.508	0.581	0.124	0.020	-0.002
F_{IS}[g]	-0.020	-0.047	-0.085	-0.033	0.056	-0.042	-0.015
McaB5							
N	111	140	36	69	20	288	89
#A	4	5	5	5	6	6	6
A_R	3.281	3.661	3.940	4.188	5.475	10.777	13.580

Table A 4.2 Continued (2)

Microsatellite	MA	DB	SC	Egulf	wGulf	Atlantic	Gulf
H_E	0.819	0.834	0.834	0.797	0.863	0.827	0.809
H_O	0.883	0.893	0.946	0.725	0.700	0.896	0.719
P_{HW}	0.540	0.310	0.307	0.061	0.194	0.005	0.182
F_{IS}^g	-0.078	-0.071	-0.135	0.090	0.189	-0.083	0.111
McaB6							
N	111	140	37	69	20	288	89
#A	7	8	3	5	4	8	5
A_R	3.235	3.638	2.559	3.934	3.872	7.993	8.320
H_E	0.336	0.348	0.252	0.664	0.662	0.330	0.659
H_O	0.198	0.207	0.135	0.435	0.450	0.194	0.438
P_{HW}	0.306	0.392	0.861	0.007	0.114	0.029	0.238
F_{IS}^g	0.410	0.405	0.464	0.345	0.320	0.411	0.335
McaB22							
N	111	140	37	69	20	288	89
#A	12	15	11	10	11	15	13
A_R	9.520	10.081	9.127	8.687	10.614	17.963	18.023
H_E	0.933	0.930	0.913	0.905	0.909	0.929	0.908
H_O	0.973	0.900	0.946	0.870	0.800	0.934	0.854
P_{HW}	0.193	0.473	0.222	0.258	0.087	-0.025	0.080
F_{IS}^g	-0.043	0.032	-0.036	0.039	0.120	-0.005	0.060
McaB26							
N	111	140	36	69	20	288	89
#A	3	3	3	3	3	3	3
A_R	2.891	2.686	2.721	2.202	2.736	6.667	5.607
H_E	0.436	0.326	0.335	0.098	0.274	0.371	0.139
H_O	0.369	0.286	0.216	0.087	0.250	0.309	0.124
P_{HW}	0.383	0.356	0.062	0.893	-0.092	0.011	-0.050
F_{IS}^g	0.152	0.125	0.354	0.115	0.087	0.166	0.110
McaB28							
N	111	140	37	69	20	288	89

Table A 4.2 Continued (3)

Microsatellite	MA	DB	SC	Egulf	wGulf	Atlantic	Gulf
#A	4	3	2	2	1	4	2
A_R	1.925	1.665	1.459	1.362	1.000	4.585	4.112
H_E	0.062	0.049	0.027	0.029	0.000	0.051	0.023
H_O	0.063	0.050	0.027	0.015	0.000	0.052	0.011
P_{HW}	0.906	0.930	1.000	1.000	NA	-0.019	0.000
F_{IS}^g	-0.019	-0.019	0.000	0.500	NA	-0.019	0.500
McaB33							
N	111	140	37	69	20	288	89
#A	5	4	4	6	3	5	7
A_R	3.934	3.909	3.983	4.583	2.773	8.402	10.282
H_E	0.522	0.506	0.535	0.453	0.267	0.515	0.414
H_O	0.505	0.543	0.595	0.420	0.300	0.535	0.393
P_{HW}	0.518	0.094	0.176	0.512	-0.123	-0.054	0.003
F_{IS}^g	0.033	-0.074	-0.112	0.072	-0.123	-0.038	0.049
McaB37							
N	111	140	37	69	20	288	89
#A	4	4	4	3	3	4	5
A_R	3.505	2.961	3.422	2.595	2.936	9.047	9.523
H_E	0.738	0.710	0.524	0.719	0.613	0.703	0.697
H_O	0.514	0.464	0.324	0.420	0.300	0.465	0.393
P_{HW}	0.549	0.472	0.391	0.183	0.265	0.019	0.171
F_{IS}^g	0.305	0.346	0.381	0.415	0.511	0.339	0.435
McaB40							
N	111	140	34	69	20	288	89
#A	3	4	4	3	2	4	3
A_R	2.283	2.350	3.000	2.605	2.000	7.876	7.796
H_E	0.475	0.514	0.665	0.634	0.405	0.521	0.598
H_O	0.378	0.486	0.432	0.420	0.400	0.438	0.416
P_{HW}	0.113	0.315	0.048	0.178	0.399	0.035	0.138
F_{IS}^g	0.204	0.055	0.350	0.337	-0.241	0.159	0.305

Table A 4.2 Continued (4)

Microsatellite	MA	DB	SC	Egulf	wGulf	Atlantic	Gulf
MaFYP							
N	110	140	37	69	20	288	89
#A	9	9	8	9	9	10	9
A_R	6.766	7.333	6.881	7.774	8.440	14.070	15.255
H_E	0.871	0.822	0.821	0.860	0.867	0.842	0.862
H_O	0.757	0.736	0.757	0.710	0.650	0.747	0.697
P_{HW}	0.060	0.094	0.234	0.090	0.216	0.062	0.115
F_{IS}^g	0.131	0.105	0.078	0.174	0.250	0.113	0.192
MaWS1							
N	111	140	37	69	20	288	89
#A	3	3	4	2	2	4	2
A_R	2.124	1.551	2.882	1.595	1.810	4.456	4.906
H_E	0.170	0.056	0.228	0.086	0.195	0.123	0.109
H_O	0.090	0.036	0.135	0.029	0.050	0.069	0.034
P_{HW}	0.815	0.964	0.864	0.987	0.000	-0.023	-0.012
F_{IS}^g	0.470	0.366	0.408	0.661	0.743	0.437	0.691
Gg16							
N	111	140	37	69	20	288	89
#A	4	6	4	4	4	6	6
A_R	3.306	3.620	3.171	3.124	3.983	7.251	9.455
H_E	0.471	0.528	0.498	0.526	0.658	0.502	0.554
H_O	0.460	0.543	0.432	0.377	0.400	0.497	0.382
P_{HW}	0.418	0.320	0.239	0.044	0.331	0.007	0.148
F_{IS}^g	0.025	-0.028	0.132	0.284	0.392	0.012	0.310