A Morphological Key to Distinguish Among Smoothhound Sharks (Genus *Mustelus*) in the Gulf of Mexico

Una Clave Morfológica para Distinguir entre Tiburones Smoothhound (Género *Mustelus*) en el Golfo de México

Une Clé Morphologique pour Distinguer les Poissons Requins (Genus *Mustelus*) dans le Golfe du Mexique

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ABSTRACT

Extensive overlap in external morphology among species of smoothhound sharks (genus *Mustelus)* has made identification of individual species difficult. Consequently, verifying the distribution of individual species in the Gulf of Mexico (Gulf) and planning effective strategies for their management and conservation are problematic. Phylogenetic analysis of sequences of the mitochondrially-encoded NADH-2 gene identified three reciprocally monophyletic lineages, which correspond to three different species of smoothhound sharks in the Gulf, a result verified via genotypes at nuclear-encoded microsatellites. When adult specimens (both sexes) were separated based on genetic characteristics, we discovered differences in external morphology that permit reliable species identification in the field. Here, we present a diagnostic key to distinguish among smoothhound sharks (*Mustelus canis, Mustelus sinusmexicanus* and *Mustelus norrisi)* in the Gulf. Results of this study should prove useful in management and conservation efforts for smoothhound sharks resources in the Gulf.

KEY WORDS: Smoothhound sharks, field key, species identification

INTRODUCTION

Species in the genus *Mustelus* are cartilaginous fishes belonging to the order Carcharhiniformes (ground sharks) and the family Triakidae (hound sharks), which is represented by 47 described species in nine genera (Eschmeyer 2012). The genus *Mustelus* (smoothhound sharks) contains 28 nominal species, many of which are difficult to discern based on external morphology (Heemstra 1997). Species of *Mustelus* are found in estuaries, coastal marine waters, and continental and insular slopes, and many species are important regional fishery resources (Castro 2011, Compagno 2005). Within the Gulf of Mexico (hereafter Gulf), there are four described species (Compagno 2005): the dusky smoothhound shark (*Mustelus canis*), the Florida smoothhound shark (*Mustelus norrisi*), the small-eye smoothhound shark (*Mustelus higmani*), and the Gulf of Mexico smoothhound shark (*Mustelus sinusmexicanus*). In recent years, there has been dissent among scientists and fisheries managers regarding the number of species of *Mustelus* in the region. Due to the inability of scientists and fishers to distinguish smoothhound species in the field, based on current morphological keys (NMFS 2010a), the National Marine Fisheries Service (NMFS) has promoted managing smoothhound sharks as a single species in U.S. waters of the Gulf (NMFS 2010a).

A reliable method for identifying species of smoothhound sharks in the field is needed to alleviate confusion and to inform efforts to map species distributions. The diagnostic morphological characters currently used to distinguish among species of *Mustelus* in the western Atlantic (position of fins, internarial distance, pattern of buccopharyngeal denticles, ridges on the dermal denticles, and labial furrow size (Heemstra 1997, Rosa and Gadig 2010) are highly variable, with considerable overlap among species (Castro 2011, Heemstra 1997, Lopez et al. 2006). Both *M. canis* and *M. sinusmexicanus* mature later and grow to larger size than either *M. higmani* or *M. norrisi* (Heemstra 1997, Compagno 2005), and while it is possible that other life-history characteristics (*e.g.*, age at maturity, maximum age, female fecundity) may differ among the species, there is a paucity of life-history data available for smoothhound sharks. Here, we provide a simple morphological key to distinguish among smoothhound species in the Gulf.

METHODS

A fin clip (~1 cm^2) was taken from the trailing edge of the first dorsal or left pelvic fin of 209 smoothhound sharks sampled between 2010 and 2012 from localities within the Gulf. Fin clips were obtained by NOAA/NMFS and several independent shark surveys. Tissue was fixed in 20% DMSO storage buffer or 95% ethanol and sent to our laboratory in College Station, Texas. Whole genomic DNA was extracted from each animal via the Chelex resin (Bio-rad®) extraction method (Estoup et al. 1996). Polymerase chain reaction (PCR) primers specific for the 1047 bp NADH-dehydrogenase subunit 2 (ND-2) region of mitochondrial (mt)DNA of *M. canis* were designed and used to amplify fragments from a subset of 40 individuals. Primer sequences were as follows: forward 5'-CCA TAC CCC AAC CAT GTG GTT-3', reverse 5'-GCT TTG AAG GCT TTT GGT CTG-3'. Amplicons were electrophoresed on 2.0% agarose gels, extracted and purified with a

QIAquick Gel Extraction Kit (QIAGEN, www.qiagen.com). PCR products were sent to the Interdisciplinary Center for Biotechnology Research at the University of Florida (http://www.biotech.ufl.edu/) for sequencing. Computer-generated sequences were corrected by eye, aligned using SEQUENCHER v. 4.8 (Gene Codes Corp.), and grouped using DNASP (Rozas et al. 2003). Phylogenetic analyses employed MEGA v. 5 (Tamura et al. 2011) to test for reciprocal monophyly among groups. A maximum-likelihood approach (Felsenstein 1981) was employed utilizing the general, time-reversible substitution model (Lanave et al. 1984, Tavare 1986); support for nodes was calculated utilizing 500 bootstrap replicates.

All 209 individuals were assayed for allelic variation at 21 nuclear-encoded microsatellites. Descriptions of individual microsatellites, PCR primers, and reaction protocols may be found in Giresi et al. (2012). Amplicons were electrophoresed on polyacrylamide gels, using an ABI 377 automated sequencer (Applied Biosystems), following manufacturer instructions. Resulting chromatograms were analyzed in GENESCAN® v. 3.1.2 (Applied Biosystems) and alleles were scored by size in base pairs (bp), using GENOTYPER®v 2.5 (Applied Biosystems). Assignment of individuals, based on multi-locus microsatellite genotypes, to various groupings employed the assignment-test approach as implemented in STRUCTURE (Pritchard et al. 2000, Falush et al. 2007).

A total of 45, smoothhound shark specimens were obtained from Florida State University, the Dauphin Island Sea Lab, the National Oceanographic and Atmospheric Association (NOAA/NMFS) fisheries laboratory in Pascagoula, and the Massachusetts Department of Marine Fisheries. After specimens were placed into discrete clades or groups, based on mitochondrial and microsatellite data, respectively (see Results), male and female specimens from each of three identified species groups (*M. canis, M. norrisi,* and *M. sinusmexicanus)* were examined to identify morphological features unique to each species. A dichotomous key was then constructed, with the intent of allowing fishers and scientists to distinguish among species, using a minimum number of easily identifiable characters.

RESULTS

Phylogenetic analyses of mitochondrially-encoded ND -2 sequences resolved three reciprocally monophyletic clades of smoothhound sharks in the Gulf (Figure 1); existence of three distinct genetic groups also was supported by assignment tests based on microsatellite genotypes (not shown). While four species of smoothhounds have been described in the Gulf (Compagno et al. 2005), only three species were represented among the 209 genetic samples utilized in the study. To identify each species group as to nominal species, the ND-2 region of a specimen of *M. canis* from Cape Cod (Massachusetts, U.S.A) was sequenced. Only *M. canis* is known from this area (Compagno et al. 2005), and the ND-2 sequence from this

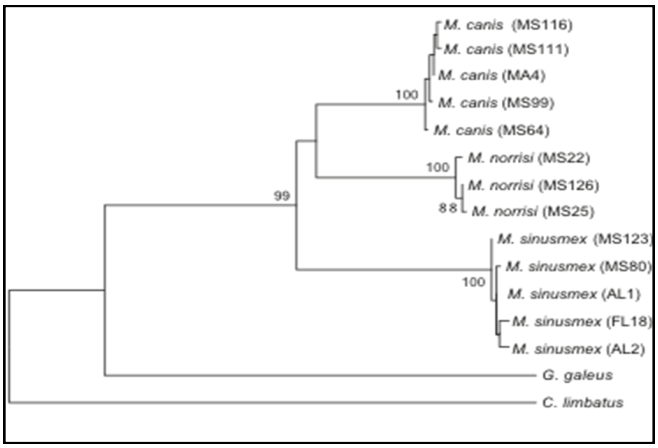

Figure 1. Phylogenetic hypothesis of three species of smoothhound sharks in U.S. waters of the Gulf of Mexico, based on sequences of the mitochondrially-encoded NADH -2 (ND2) gene. Outgroups are the triakid *Galeorhinus galeus* and the carcharhinid *Carcharhinus limbatus*. Numbers next to bifurcating branches represent bootstrap support values in percent (of 500 replicates); only values greater than 75 (%) are shown. Numbers in parentheses next to taxa names represent the specimen identification numbers used to distinguish among individuals.

individual fell within one of the three clades identified from the Gulf. This specimen was kept for morphological assessment. The clade identified as *M. norrisi* was identified by the small size of sexually mature males (Bigelow and Shroeder 1963, Heemstra 1997). *Mustelus sinusmexicanus* was identified as the third clade, based on the large size of the specimens in this group and the species description in Heemstra (1997). Based on the genetic data, 125 specimens were identified as *M. canis,* 24 specimens were identified as *M. norrisi*, and 60 specimens were identified as *M. sinusmexicanus*. *Mustelus higmani* was not represented among the specimens examined.

A small number of morphological characters were identified that unambiguously distinguished the three species. First, the upper labial furrows of *M. sinusmexicanus* were noticeably longer than the lower labial furrows (Figure 2); whereas the upper and lower labial furrows in both *M. canis* and *M. norrisi* were comparable in size (Figure 2). Second, the ampullae of Lorenzini (hereafter ampullae) lateral to the labial furrows of all three species are biserial. However, immediately posterior to the labial furrows, the ampullary series remain biserial in *M. sinusmexicanus,* but become uniserial in both *M. canis* and *M. norrisi* (Figure 2). Third, the lower lobe of the caudal fin in *M. norrisi* is pointed and directed posteriorly (as indicated by both Bigelow and Shroeder 1963 and by Heemstra 1997); whereas the lower lobe of the caudal fin is rounded in both *M. sinusmexicanus* and *M. canis* (Figure 3). Fourth, the posterior margin of the pectoral and pelvic fins of *M. canis* are nearly straight when the animals are laid flat; whereas the posterior margin of the pectoral and

pelvic fins of *M. sinusmexicanus* and *M. norrisi* are falcate (Figure 4). Fifth, the anterior nasal flaps of *M. canis* are wide (expanded medially); whereas the anterior nasal flaps are narrow and not expanded medially in *M. norrisi* and *M. sinusmexicanus* (Figure 2). Finally, *M. canis* and *M. sinusmexicanus* reach maturity at much larger sizes than *M. norrisi*; males of *M. norrisi* reach sexual maturity at 57-61cm total length, while males of *M. canis* and *M. sinusmexicanus* reach maturity at 80 cm or greater (Heemstra 1997). If a male smoothhound is less than 80cm and has calcified claspers, it is almost certainly *M. norrisi.* These discriminating characters were used to create a field key (Appendix 1) to distinguish among these three species of *Mustelus*.

DISCUSSION

Here, we present a reliable key to distinguish among three smoothhound shark species (*M. canis*, *M. norrisi*, and *M. sinusmexicanus*) in the U.S. waters of the Gulf of Mexico. These easily ascertained characters will allow fishers to distinguish among the three species expeditiously and accurately, leading to a better understanding of the relative abundance and distribution of smoothhound species in the Gulf of Mexico. Other characters, such as the presence of mostly tridentate dermal denticles on the flank of *M. sinusmexicanus* versus mostly lanceolate denticles in *M. canis* and *M. norrisi* (Heemstra 1997) may be useful in distinguishing among species, but require the use of a microscope, which typically is rare on board fishing vessels.

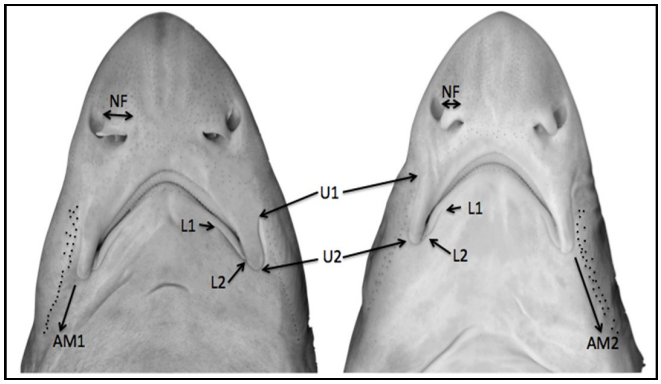

Figure 2. Differences on the ventral surface of the head among species of *Mustelus* in U.S. waters of the Gulf of Mexico. The specimen on the left is *M. canis.* The specimen on the right is *M. sinusmexicanus.* **NF** represents the anterior nasal flaps –the flaps are much wider in *M. canis* than in *M. sinusmexicanus.* **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, and **U2** is the posterior bound of the upper labial furrow. The upper labial furrows of both *M. canis* and *M. norrisi* are nearly the same size or slightly longer than the lower labial furrows. In *M. sinusmexicanus,* the upper labial furrows extend anteriorly so that they are in some cases double the length of the lower labial furrows. **AM** represents the ampullae of Lorenzini directly posterior to the upper labial furrows. **AM1** shows that there is one row of ampullae in *M. canis* and in *M. norrisi,* while **AM2** shows that there are two rows of ampullae in *M. sinusmexicanus.*

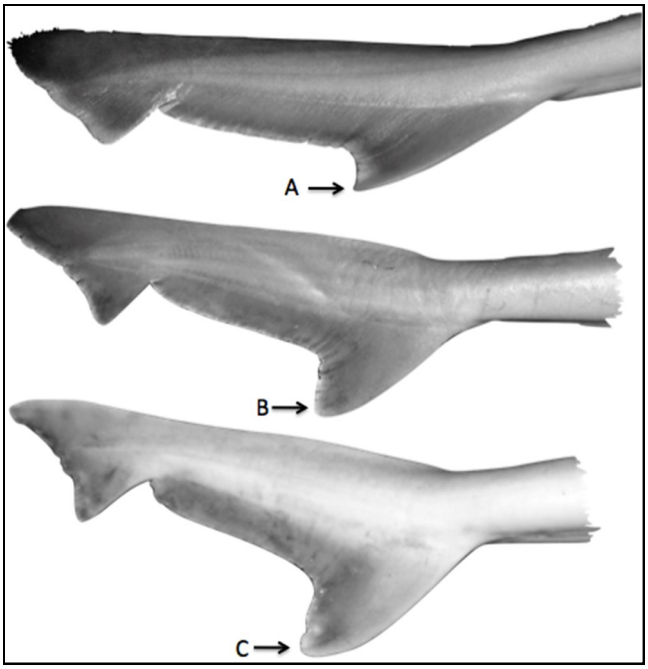

Figure 3. Differences in the lower lobe of the caudal fin of smoothhound sharks in U.S. waters of the U.S. Gulf of Mexico. Letters **A**, **B**, and **C** point to the posterior edges of the lower lobe of the caudal fin in *M. norrisi, M. canis*, and *M. sinusmexicanus*, respectively. **A** is slightly falcate and directed backwards. **B** is nearly straight with a rounded tip. **C** is falcate with a rounded tip and angled backwards.

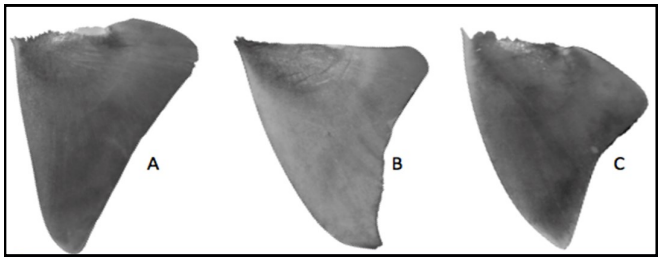

Figure 4. Comparison of pectoral fin shape among smoothhound sharks in U.S. waters of the U.S. Gulf of Mexico. Insertion to body is located at the top left corner of each fin, posterior margin of pectoral fin is the rightmost edge, nearest to letter. **A** is the pectoral fin of *M. canis,* with a nearly straight posterior margin. **B** is the pectoral fin of *M. sinusmexicanus* with a falcate posterior margin. **C** is the pectoral fin of *M. norrisi* with a falcate posterior margin.

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Appendix 1:

Field Key to Distinguish Among Smoothhound Sharks in the U.S. Gulf of Mexico

1a. Upper labial furrow noticeably longer than lower labial furrow, ampullae adjacent to upper labial furrow biserial posterior to corner of mouth, margin of lower lobe of caudal fin curved with a rounded lobe, males mature greater than 80cm total length . . . . . ***M. sinusmexicanus***

1b. Upper labial furrow slightly longer than or the same size as lower labial furrow, ampullae adjacent to upper labial furrow uniserial posterior to corner of mouth . . . . . **Go To 2**

2a. Margin of lower lobe of caudal fin nearly straight with a rounded lobe, pectoral fin free rear tips broadly rounded, posterior margins of pectoral and pelvic fins nearly straight, males mature greater than 80 cm total length . . . .***M. canis***

2b. Lower lobe of caudal fin pointed and directed posteriorly, pectoral fin free rear tips angular to narrowly rounded, posterior margins of pectoral and pelvic fins falcate, males mature less than 75 cm length. . . . . .. …..***M. norrisi***