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Píndaro Díaz-Jaimes, Douglas H. Adams, Nadia S. Laurrabaquio-Alvarado, and Elena Escatel-Luna

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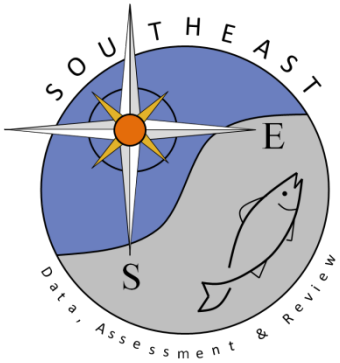

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# Preliminary mtDNA assessment of genetic stock structure of the bonnethead, *Sphyrna tiburo*, in the eastern Gulf of Mexico and northwestern Atlantic

Píndaro Díaz-Jaimes[a]

Douglas H. Adams[b]

Nadia S. Laurrabaquio-Alvarado[a]

and

Elena Escatel-Luna[a]

[a] Laboratorio de Genética de Organismos Acuáticos, Instituto de Ciencias del Mar y Limnología, Universidad Nacional Autónoma de México. Apdo. Postal 70-305, México D.F., 04510, México.

[b] Florida Fish and Wildlife Conservation Commission, Fish and Wildlife Research Institute, 1220 Prospect Avenue, Suite 285, Melbourne, Florida, 32901, USA.

**Abstract**

Bonnethead sharks in U.S. waters are currently managed as one population or stock, although the existence of multiple genetically distinct stocks has not been addressed using molecular techniques. Additional information regarding genetic stock delineation is critical for effective management and conservation. The present study provides preliminary results from an ongoing study to evaluate the genetic population structure of bonnethead sharks in the Gulf of Mexico and U.S. south Atlantic. A total fragment of 940 base pairs of the mtDNA-control region was sequenced in 140 bonnethead samples resulting in a high mean haplotype and nucleotide diversity (h=0.8817; $\pi$=2.27%) when compared with other shark species. Genetic diversity was typically lower for locations from estuaries known to be nursery grounds. Significant spatial genetic differences were observed among bonnetheads from the Gulf of Mexico and U.S. south Atlantic ($\Phi_{CT}$ = 0.0558; P = 0.033), suggesting restricted gene flow between the two areas. Although current data indicate genetic differences exist between bonnetheads inhabiting the Gulf of Mexico and U.S. south Atlantic, these results are preliminary and further analyses from this ongoing study will provide a better understanding of population structure in these two large marine systems.

## Introduction

Bonnethead, *Sphyrna tiburo*, are commonly distributed in the western Atlantic from North Carolina, U.S. to southern Brazil, including the Gulf of Mexico and the Caribbean, and are seasonally found within estuarine, coastal, and continental shelf waters (Compagno, 1984). There have been no observations of bonnetheads moving between the Gulf of Mexico and waters of the U.S. south Atlantic (non-Gulf waters)(Kohler and Turner, 2007).

Although the continuous distribution of populations across the species range in Florida waters may infer the existence of a single panmictic population, regional differences in life history parameters of bonnetheads have been observed which suggest there may be multiple populations within the range of this species. For example, latitudinal differences in adult and embryo size, length and age at maturity, and growth rates were found between three different Florida Gulf coast regions (northwest Florida, Tampa Bay, Florida Bay) (Parson 1993; Lombardi-Carlson, 2003). There have also been significant differences in the concentration of thyroidal hormone from maternal serum and embryo yolk tissue between Florida Bay and Tampa Bay estuaries (McComb *et. al.*, 2005). These regional/latitudinal differences may be environmentally controlled or physiologically influenced, but genetic factors may also play a role (Conover, 1990). To date, no studies have been conducted to confirm if these differences are related to a genetic divergence of populations, and the stock structure of bonnetheads has not been fully assessed within its range. Bonnetheads in U.S. waters are currently managed as one population or stock. Genetic data for this species is lacking and the existence of multiple genetically distinct stocks is possible. More comprehensive information regarding genetic stock delineation is

critical for effective management and conservation. For this purpose, the use of molecular markers based on both mitochondrial and nuclear DNA, are useful to address these questions. Therefore, the present study is directed to evaluate the genetic population structure of bonnetheads between areas showing differences in biological parameters in the Gulf of Mexico and U.S. south Atlantic for this small coastal species. Preliminary mitochondrial DNA results are presented in this report and nuclear DNA results are currently being finalized.

**Materials and Methods**

Bonnetheads were collected from 1992 to 2013 by the Florida Fish and Wildlife Conservation Commission – Fish and Wildlife Research Institute's (FWC-FWRI) Fisheries-Independent Monitoring Program operating in estuarine systems and adjacent coastal waters, or from recreational and commercial fisheries in the nearshore and offshore waters of the U.S. south Atlantic (referred to hereafter as Atlantic) and the Gulf of Mexico in the southeastern U.S.

Atlantic region study areas ranged from waters near the Georgia-Florida border south to the Florida Keys area. Specific estuarine study areas within the Atlantic region included the St. Marys River system, the Nassau Sound - Nassau River system, the lower St. Johns River system, Indian River Lagoon, Florida Keys, and waters adjacent to these areas. Within Gulf of Mexico waters, bonnetheads were collected from Cedar Key, Tampa Bay, Charlotte Harbor, and from nearshore and offshore waters adjacent to these areas within the West Florida Shelf. A limited number of samples were also collected from Apalachicola Bay in this region.

Bonnetheads were collected from using 183-m center-bag haul seines (37.5 mm stretch mesh), monofilament gillnets (stretch mesh sizes ranging from 50 mm to 152 mm), and hook and line methods (Adams and McMichael, 1999; Kupschus and Tremain, 2001; Adams and Paperno, 2007). Fish were placed directly on ice after capture and returned to the laboratory or were processed in the field; species, precaudal length (PCL), fork length (FL), total length (TL), and sex were recorded. When possible, stage of maturity for females was determined by macroscopic examination of the reproductive tract. Maturity in males was determined by length and calcification of claspers. Umbilical scars were characterized as "umbilical remains," "fresh open," "mostly healed," "partially healed," and "none" according to Pratt, et al. (1998). Dorsal muscle and fin clips from each specimen were collected and preserved in ethanol.

Total genomic DNA was isolated using Wizard Genomic® Promega kit and resuspended in 50 - 100 μL of TE buffer. A fragment of 940 base pairs (bp) of the mitochondrial control region of bonnetheads was amplified in 140 samples by using the primers ElasmoCR15642F (5'-TTGGCTCCCAAAGCC-3') and ElasmoCR16638R (5'-CCCTCGTTTTWGGGGTTTTTCGAG-3'; Stoner et al. 2003). Reactions for sequencing were done in a total volume of 50 μL containing 50-100 ng DNA, in amplification buffer, 10 mM TRIS-HCl (pH 8.4), 50 mM KCl, 1.5 mM MgCl2, 0.2 mM of each dNTP, 0.1mM of each primer and 2.5 units of platinum Taq DNA polymerase (Invitrogen, Cat. 10966-030). PCR amplifications consisted of 35 cycles of 1 min at 95ºC for denaturalization, 1 min at 59ºC for annealing, and a final extension at 65ºC for 3 min. PCR products were sequenced in the forward direction on an ABI 3730xl automated sequencer applying the dye-termination method (Applied Biosystems).

Multiple alignment was performed with Clustal X ver. 1.8 (Thompson et al. 1997) as well as by optimizing the gap penalties in order to minimize artificial homologies between haplotypes (homoplasy) during the alignment. The hierarchical likelihood ratio method implemented in jModelTest 2.0 (Darriba et al. 2012) was applied to define the most appropriate substitution model of sequence evolution for the segment of the mtDNA-control region analyzed

Haplotype (h) and nucleotide ($\pi$) diversities were estimated using Arlequin 3.5 (Excoffier and Lischer, 2010) with the application of the Tamura-Nei substitution model. The molecular analogues of the unbiased Wright's *F*-statistics ($\Phi$-statistics) were computed in hierarchical analyses of molecular variance (AMOVA), and pairwise $\Phi_{ST}$ between localities were obtained to identify genetically differentiated localities. AMOVA was used to test panmixia among samples grouped into Atlantic and Gulf of Mexico regions and differentiation was assessed among ($\Phi_{CT}$) and within ($\Phi_{SC}$) coasts, with Florida Keys being considered as an intermediate area for both regions.

**Results and Discussion**

A total fragment of 940 base pairs of the mtDNA-control region was sequenced in a total of 140 samples of bonnetheads resulting in 49 haplotypes. Additional samples are currently being analyzed. We observed 34 segregating sites featuring 23 transitions and 12 transversions. As a result, the mean haplotype diversity was high (h=0.8817) when compared with other shark species, with values ranging from 0.8206 in Charlotte Harbor to 1.0 for offshore Gulf of Mexico and the intermediate or potential mixing area of the Florida Keys. The mean nucleotide diversity was $\pi$=2.27%, ranging from 1.6% for Charlotte

Harbor to 3.4 for the offshore Gulf of Mexico location. Genetic diversity was typically lower for locations from estuaries known to be nursery grounds (Table 1).

Significant spatial genetic differences were observed from the AMOVA among samples grouped into Atlantic and Gulf of Mexico ($\Phi_{CT}$ = 0.066; P = 0.033), suggesting restricted gene flow between the two regions. Differences within samples in each regions were lower than among regions ($\Phi_{SC}$ = 0.0224; P=0.048) suggesting genetic homogeneity within each region (Table 2).

Current data suggest potential differences between Gulf of Mexico and Atlantic bonnethead populations, but further analyses is required to better understand population structure in these two large marine systems. The genetic differences observed ($\Phi_{CT}$ = 0.0558; P = 0.033) are comparatively lower than reports for other shark species where the same molecular marker was used for samples from the northwestern Atlantic and western Florida. For example, levels of genetic divergence between blacktip shark, *Carcharhinus limbatus*, neonates from nursery areas along the Gulf of Mexico coast of Florida (Yankeetown, Terra Ceia Bay and Pine Island Sound) and northeast Atlantic (Bulls Bay, S.C.) were higher ($\Phi_{CT}$=0.090, P<0.001) than the values reported here (Keeney et al. 2003). Thus, the differences observed should be interpreted cautiously, and may be influenced by the existence of philopatry in the bonnetheads. Therefore, additional genetic evidence is necessary to confirm or discard the existence of two genetically discrete populations of bonnetheads in Gulf of Mexico and Atlantic waters. We are currently analyzing additional samples from the Gulf of Mexico and Atlantic regions, including samples from Mexican waters, to better understand population structure and to examine philopatry in this species. Additionally, we are examining nuclear DNA markers for expanded resolution regarding

the two-stock hypothesis, as well as further define the potential existence of philopatry in bonnetheads within waters of the U.S. and Mexico.

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Table 1. Control region-mtDNA sequence variability of bonnethead samples from the eastern Gulf of Mexico and northwestern Atlantic. Sample size (*n*), number of haplotypes (nh), haplotype diversity (h), nucleotide diversity ($\pi$), mean pairwise differences between individuals (k). (CK = Cedar Key, FL; CH = Charlotte Harbor, FL; OFF_GM = offshore Gulf of Mexico; NEATL = northeastern Florida; CATL = central east coast of Florida; FK = Florida Keys).

| Collection | *n* | *nh* | *h* | $\pi$ | k |
|---|---|---|---|---|---|
| CK | 35 | 16 | 0.847 | 0.0023 | 10.8 |
| CH | 32 | 10 | 0.821 | 0.0016 | 4.6 |
| OFF_GM | 13 | 13 | 1.000 | 0.0034 | -- |
| NEATL | 43 | 18 | 0.846 | 0.0022 | 11.1 |
| CATL | 12 | 6 | 0.848 | 0.0018 | 4.1 |
| FK | 5 | 5 | 1.000 | 0.0023 | -- |
| Total | 140 | 49 | 0.882 | 0.0023 | 26.4 |

Table 2. AMOVA analysis for spatial genetic variation of mtDNA-control region sequences for bonnethead samples from Atlantic and Gulf of Mexico.

| Source of variation | % of Variation | Fixation index | F statistics | P |
|---|---|---|---|---|
| Among groups (Atlantic-Gulf of Mexico) | 6.61 | $\Phi_{CT}$ | 0.066 | 0.033 |
| Among populations within groups | -0.0 | $\Phi_{SC}$ | -0.00006 | 0.468 |
| Within populations | 93.4 | $\Phi_{ST}$ | 0.066 | 0.004 |