2017 SMALL GRANT IN AID OF RESEARCH – ANNUAL SUMMARY REPORT

Genetic characterization of the Antillean manatee population in Cuba: implications for the conservation of the species.

Anmari Alvarez Aleman

School of Natural Resources and Environment, University of Florida

The general goal of my research was the use of molecular approaches to address important conservation-oriented research questions in order to generate scientific knowledge about West Indian manatee (*Trichechus manatus*).

For this work, tissues from 82 specimen corresponding to loose bones, carcass, museum specimens and live manatees, were collected from the north and south of Cuba. The DNA was extracted using different techniques aiming for the best quality (concentration, 260/280 and 260/230). These techniques were phenol/chloroform extraction, with and without the use of phase lock gel tubes, QIAGEN's DNeasy blood and tissue kit and SPRI beads (Solid Phase Reversible Immobilization).

The primers CR4 and CR5 from Garcia-Rodriguez et al. (1998) were used to amplify the mitochondrial DNA control region displacement loop segment from Cuban samples (Garcia-Rodriguez et al. 2000, Pause et al. 2007, Nourisson et al. 2011). The PCR amplifications were carried out with the protocol proposed by Kellogg (2008). Up to date we have been able to obtain 58 sequences from Cuba. The analysis of these sequences revealed a total of three haplotypes for the Cuban manatee population. Haplotypes A01, A03 and a new haplotype for the species (that hasn't been named yet). Haplotype A01 was the dominant in this population. The haplotype diversity (Hd) was 0.068, nucleotide diversity (π) was 0.00025 and the number of polymorphic sites (S) was 3. These indexes are low when comparing with other populations of the species.

The Sequence from the Cuban population of manatees will be compared with 430 control region mtDNA sequences of *T. manatus* from Florida, Puerto Rico, Dominican Republic, Mexico, Belize, Panama, Colombia, Venezuela, Guyana and Brazil. These last sequences were downloaded from GenBank and corresponded to each of the manatee haplotypes described in the literature from 1998-2012. Haplotype frequencies were also obtained either from the literature or by contacting the corresponding authors.

Additionally, 18 polymorphic loci available for manatees were used to genotype individuals from Cuba (Garcia-Rodriguez et al. 2000, Pause et al. 2007, Tringali et al. 2008). I was able to completely genotype 36 Cuban manatees with 16 loci. I continue doing amplifications attempts

since some of the samples were in very bad condition. The number of alleles per locus in the current data set ranged from 2 to 6. The average number of allele (Na) was 3 and the expected heterozygosity (He) was 0.46.

For future analysis I will compare the genotypes observed in Cuba with individuals from Florida, Puerto Rico and Belize. Microsatellite genotypes from Florida, Belize and Puerto Rico are going to be kindly provided by USGS-Sea to Shore Alliance and FWC (Tringali et al. 2008, Tucker et al. 2010, Hunter et al. 2010, Hunter et al. 2012).

Furthermore, I'm going to implement GBS analysis with the Cuban samples using previous protocols (Wallace and Mitchell unpublished, Elshire et al. 2011). I will use PstI, a six base cutter, methylation sensitive restriction enzyme, to reduce the genome complexity. The fragments obtained after the DNA digestion will be sequenced in order to explore for the presence of SNPs (Elshire et al. 2011). The polymorphisms identified, via bioinformatics post-processing using Stacks or GBS-SNP-CROP analysis pipelines, will be used to deeply evaluate within population diversity (Elshire et al. 2011, Catchen et al. 2013, Melo et al. 2016) and explore putatively adaptive divergence.

I am currently finishing the amplification of some samples that required further amplification, and the primary and statistical analysis to reveal the genetic structure and answer initial research questions. These results will be included in my PhD which I'm planning to defend in late 2019. The results of my work will be published later in scientific journals.

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