

# Description of mtDNA Markers of Loggerhead Marine Turtles from Caribbean Colombia

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The loggerhead sea turtle (*Caretta caretta*) occurs throughout tropical, subtropical and temperate waters (Bolten & Witherington 2003). Once considered to be a nesting population of ~500 females, currently the loggerhead rookery along the Caribbean coast of Colombia is reported to be greatly reduced in size (Amoroch 2003). This population in Northern Colombia is considered to be part of the Northwestern Atlantic regional population (Conant *et al.* 2009), although detailed population structure data, based on molecular markers, are lacking from loggerheads that occur here. As a first step toward elucidating the relationship between loggerheads from Colombia and those from throughout the Wider Caribbean, we conducted preliminary assessments of mitochondrial DNA (mtDNA) haplotypes of nesting and foraging loggerhead turtles sampled in Colombia, using direct sequencing and phylogenetic inference. During 2008 and 2009, we collected blood samples from the dorsal cervical sinus from eight loggerhead turtles of the Colombian Caribbean: five nesting females from Don Diego beach (11°16'N, 73°45'W) and three juvenile turtles captured while they were foraging around San Martín de Pajarales Island (10°11'N, 75°47'W). Genomic DNA was extracted from each blood sample using a commercial kit (UltraClean™ Tissues & Cells, MO BIO Laboratories, Inc, California, USA). The mtDNA control region was PCR-amplified using primers TCR-5 and TCR-6 (Norman *et al.* 1994). PCR products of control region (mtDNA) with a product size of ~398 bp were obtained. Subsequently these products were purified and directly sequenced at Macrogen Inc. (Seoul, South Korea). A basic local alignment search (BLAST-GenBank) was performed to identify the haplotypes in Colombian aggregations. To perform the phylogenetic inference, the sequences were assembled and aligned (Hall 1999), and phylogenetic analysis was performed using maximum parsimony or MP (PAUP 3.0, Swofford 1991) and maximum-likelihood or ML criteria (Rax-ML, Cipress 2.0, Stamatakis *et al.* 2005). To provide statistical support for the phylogenetic trees, we developed a bootstrap analysis (Felsenstein 1985).

Two previously reported haplotypes were identified in the Colombian samples. Haplotype CC-A1, which is present in nesting colonies of North and South Carolina, Georgia and NE Florida with very high frequencies (>80%)(USA) was found in four of our samples. Haplotype CC-A2, found in three of our samples, was previously reported as the dominant haplotype for Quintana Roo (Mexico) and South Florida rookeries (SE and SW combined), as well as being the most frequent haplotype among analyzed Cuban turtles (Ruiz-Urquiola *et al.* 2010). Prior studies have reported a cline of these main haplotypes, with decreasing frequencies of haplotype CC-A1 and increasing frequencies of CC-A2 from north to south (Encalada *et al.* 1998; Bowen *et al.* 2005 and Shamblin *et al.* 2011). A new sequence, labeled as CC-SM1 was also found in one individual (Table 1) sampled at the Don Diego nesting beach. This haplotype is similar to CC-A1 (90%), but has a total of seven polymorphic sites consisting of insertion/deletions (indels).

Phylogenetic analysis (MP and ML) revealed a relationship between the nesting and feeding aggregations of Colombia with major populations in the Atlantic and Mediterranean. The nesting aggregation in Colombia is related to nesting colonies in South Florida (USA) and Mexico. Loggerhead turtles from the foraging area around San Martín de Pajarales are grouped with other aggregations of feeding populations from the North Atlantic, Mediterranean Sea (Spain and Italy) and to sequences frequently reported from nesting populations in the North Atlantic and Mexico. This pattern suggests that individuals that use the Colombian Caribbean for feeding and reproductive activities are part of an Atlantic meta-population, where sequences CC-A1 and CC-A2 are the most frequent haplotypes. The Southeastern USA and Mexico loggerhead populations may be the sources of the nesting aggregation in Colombia by means of recent colonization events (Bowen & Karl 2007), assisted by the strong migratory behavior of loggerhead turtles and marine currents such as the Gulf Stream and the North Atlantic Gyre, but inputs from other small rookeries in the Caribbean cannot be discounted. The new loggerhead haplotype discovered in the Colombian Caribbean may be endemic to this rookery, and thus may suggest that Colombian loggerheads display natal homing.

Maximum likelihood mixed stock analysis has been used for identifying proportions of immature loggerhead turtles in developmental habitats. It has been demonstrated that their distribution is not random, but rather influenced by nearby nesting populations (source rookeries). The juveniles from the San Martín de Pajarales feeding ground (Colombian Caribbean), with haplotypes CC-A1 and CC-A2, represent the main haplotypes found in nesting rookeries throughout the Atlantic-Mediterranean system, but there is also a possibility that there are contributions from rookeries in Cuba, from the Cape Verde Islands (Monzón-Arguello *et al.* 2009), and from the coast of Africa (Carreras *et al.* 2006). The closest study

Sampling location	Life stage	Haplotypes
Don Diego Beach	Adult Female	CC-A1 (n = 2)
		CC-A2 (n = 1)
		CC-SM1 (n = 1)
San Martín de Pajarales Island	Juvenile	CC-A1 (n = 1)
		CC-A2 (n = 2)

**Table 1.** Mitochondrial DNA (mtDNA) haplotypes identified in loggerhead turtles from the Colombian Caribbean..

location (to Colombia) for loggerhead juveniles was at Chiriquí Lagoon (Panama Caribbean), where approximately 65–70% of loggerheads were shown to have originated from South Florida and Mexico nesting beaches. However, there were still unknown haplotypes in the feeding ground that were not linked to any source rookery, suggesting that there are small and un-surveyed beaches that remain to be assessed and included in these evaluations.

These data represent a first step toward elucidating the population genetic structure and phylogeography of both nesting and foraging loggerhead turtles in Colombia within the Wider Caribbean region. We plan to collect more samples over a wider geographic area for future analyses, so that we may better understand the genetic structure of these populations in the region. Further studies of loggerhead population structure is required, and we suggest a larger sample of individuals on nesting and feeding grounds, along with longer haplotypes sequences (880 bp) using primers LCM15382 and H950g that might increase the resolution of the analysis (Abreu-Grobois *et al.* 2006). The current, widely used mtDNA D-loop PCR primers generate segments of about 380 to 510 bp and, although effective in distinguishing major rookeries in earlier genetic surveys, they may have become limited in the level of resolution between rookeries as the number of candidate source rookeries has increased and the amount of haplotype frequency overlap has become more widespread.

**Acknowledgements:** We want to thank all the team from El Rodadero Aquarium Museum for logistical help in the sampling of the turtles from Don Diego Beach, and Rafael Vieira, the director of CEINER Research Center and Sea Aquarium (Corales del Rosario and San Bernardo National Park). Samples were collected under the Genetic Resources Permit granted by the Ministerio de Medio Ambiente y Desarrollo Territorial (4120-E1-45179 del 13/04/11 COR 1593-11 / RGE 0095). We also thank two anonymous reviewers for help in editing the manuscript.

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