Pontificia Universidad JAVERIANA Bogotá

Univ. Sci. 23 (3): 355-381, 2018.
doi: 10.11144/Javeriana.SC23-3.cccl

ORIGINAL ARTICLE

Complete Colombian Caribbean loggerhead turtle mitochondrial genome: tRNA structure analysis and revisited marine turtle phylogeny

Katherin Otálora[1], **Javier Hernández-Fernández**[1,*]

Edited by
Juan Carlos Salcedo-Reyes
(salcedo.juan@javeriana.edu.co)

1. Facultad de Ciencias Naturales e Ingeniería. Grupo de Investigación en Genética, Biología Molecular y Bioinformática – GENBIMOL, Universidad Jorge Tadeo Lozano, Cra 4 No 22-61, Bogotá, Colombia, South America.

* javier.hernandez@utadeo.edu.co

Received: 02-10-2017
Accepted: 16-04-2018
Published on line: 31-10-2018

Citation: Otálora K, Hernández-Fernández J. Complete Colombian Caribbean loggerhead turtle mitochondrial genome: tRNA structure analysis and revisited marine turtle phylogeny, *Universitas Scientiarum*, 23 (3): 355-381, 2018. doi: 10.11144/Javeriana.SC23-3.cccl

Funding:
Research, Creativity and Innovation Department at Universidad Jorge Tadeo Lozano.

Electronic supplementary material:
Supp. 1.

Abstract

The loggerhead marine turtle, *Caretta caretta*, is a widely distributed and endangered species that is facing critical population decline, especially in Colombian Caribbean rookeries. Mitochondrial DNA sequence data are of great importance for the description, monitoring, and phylogenetic analyses of migratory turtle populations. In this study, the first full mitochondrial genome of a loggerhead turtle nesting in the Colombian Caribbean was sequenced and analyzed. This mitochondrial genome consists of 16 362 bp with a nucleotide composition of T: 25.7 %, C: 27 %, A: 35 % and G: 12 %. Sequence annotation of the assembled molecule revealed an organization and number of coding and functional units as reported for other vertebrate mitogenomes. This Colombian loggerhead turtle (Cc-AO-C) showed a novel D-Loop haplotype consisting of thirteen new variable sites, sharing 99.2 % sequence identity with the previously reported Caribbean loggerhead CC-A1 D-Loop haplotype. All 13 protein-coding genes in the Cc-AO-C mitogenome were compared and aligned with those from four other loggerhead turtles from different locations (Florida, Greece, Peru, and Hawaii). Eleven of these genes presented moderate genetic diversity levels, and genes COII and ND5 showed the highest diversity, with average numbers of pair-wise differences of 16.6 and 25, respectively. In addition, the first approach related to t-RNAs 2D and 3D structure analysis in this mitogenome was conducted, leading to observed unique features in two tRNAs ($tRNA^{Trp}$ and $tRNA^{Leu}$). The marine turtle phylogeny was revisited with the newly generated data. The entire mitogenome provided phylogenetically informative data, as well as individual genes ND5, ND4, and 16S. In conclusion, this study highlights the importance of complete mitogenome data in revealing gene flow processes in natural loggerhead turtle populations, as well as in understanding the evolutionary history of marine turtles.

Keywords: Mitogenome; *Caretta caretta*; Cheloniidae; coding genes; sea turtle phylogeny.

Introduction

Marine turtles (superfamily Chelonioidae) comprise seven existing species grouped into two families: Cheloniidae, including the flatback (*Natator depressus*), olive ridley (*Lepidochelys olivacea*), Kemp's ridley (*Lepidochelys*

kempii), loggerhead (*C. caretta*), hawksbill (*Eretmochelys imbricata*), and green turtle (*Chelonia mydas*) species (Pritchard & Mortimer, 1999); and Dermochelyidae which currently comprises a single species, the leatherback sea turtle (*Dermochelys coriacea*).

The loggerhead turtle, *Caretta caretta* (Cc) is distributed around the oceans of the world in tropical and subtropical latitudes (Amorocho, 2003). Its main nesting locations have been reported in the coasts of the peninsula of Florida (FWC 2015), in the western Brazilian Atlantic Ocean, in the Eastern Mediterranean Sea, in the Omani Arabian Sea, in Madagascar, and in Japan (Dodd 1988, Lancheros & Hernández 2013, Hernández *et al*. 2017). Despite its wide global distribution, it is considered as an endangered species (IUCN 2016). Loggerhead populations are directly threatened by several anthropic activities including: fisheries bycatch, excessive fishing/hunting, and illegal trade of eggs and meat. In addition, Loggerhead turtle populations are affected by habitat deterioration, coastal development, pollution, pathogens and climate change (Eckert *et al*. 2000, Lancheros & Hernández, 2013, Machado & Bermejo, 2012). Loggerhead turtles reach their sexual maturity at around 20-30 years of age (Machado & Bermejo, 2012), which does not offset the rampant overall population decline of the species. The threat to Loggerhead turtles has been well documented the Colombian Caribbean (Amorocho, 2003, Ceballos-Fonseca, 2004), where the world's second highest number of catches per year (approximately 600 turtles) has been reported (Humber *et al*. 2014). This, despite existing national laws and international agreements to protect the species from anthropic threats (SWOT 2012, IUCN 2016).

In all vertebrate taxa, the mitochondrial genome (mitogenome) is arranged as a single, circular, and haploid DNA molecule that features a uniquely high mutation rate, is non-recombining, maternally inherited, and has a specific organization and expression mode (Avise, 1994). Stretches from the mitogenome constitute the most commonly used molecular markers for genetic analysis of loggerhead turtle populations (Drosopoulou *et al*. 2012, Duchêne *et al*. 2012). The loggerhead turtle mitogenome contains 37 coding units including two ribosomal RNAs (rRNAs) genes, 22 transfer RNAs (tRNAs) genes, 13 protein-coding genes, and one non-coding region of approximately 1 100 bp called the D-Loop or control region. This D-Loop contains the origin of the H replication strand and signals for mitochondrial replication and transcription (Drosopoulou *et al*. 2012, Duchêne *et al*. 2012, Chiari *et al*. 2012).

In sea turtles, as well as in other vertebrates, point mutations in tRNA genes are likely to alter the 3D structure and function of this machinery, hence compromising peptide synthesis and possibly leading to systemic lifespan-threatening conditions. Despite the key role of mitochondrial

tRNAs, their study has almost exclusively been undertaken in humans (MITOMAP, 2018). But, the availability of large databases containing thousands of tRNA sequences from hundreds of complete genomes has promoted the development of the new field of "tRNAomics" (Marck & Grosjean, 2002). Furthermore, the understanding of sea turtle tRNA secondary and tertiary structures can benefit greatly from such structural biology resources (Popenda *et al*. 2012).

Mitochondrial D-Loop haplotypes have been useful in the identification of sea turtle individuals, nesting colonies, and their relationship with foraging areas. Studies have been carried out with mitochondrial haplotypes addressing important aspects of the phylogeography, phylopathy (natal homing), genetic structure, and maternal lineages of loggerhead turtles (Bowen *et al*. 1995, Bowen & Karl, 2007). The most thorough analyses of loggerhead turtle nesting colonies, based on D-Loop sequence data, have been carried out in Brazil (Reis *et al*. 2010), Southeastern United States (Francisco *et al*. 1999), the Atlantic-Mediterranean (Encalada *et al*. 1998), and the Pacific Ocean (FitzSimmons *et al*. 1996, Nobetsu *et al*. 2004 and Hatase *et al*. 2002).

Several studies have employed data from single mitochondrial regions (*e.g*. the Cytochrome b gene (Cytb), ND4 gene, and the D-Loop) to best explain the phylogeny of different animal taxa (Dutton *et al*. 1996, Scotto 2006, Adebambo 2009, Duchêne *et al*. 2011). However, there is an ongoing debate whether single mitochondrial markers are ideal to trace phyologentic histories (Scotto, 2006). The entire mitogenome is becoming the marker of choice for phylogenetic reconstructions, since it provides better phylogenetic resolution and precision than single traditional markers (Duchêne *et al*. 2011, Novelleto *et al*. 2016, Miya *et al*. 2003, Kim *et al*. 2005, Jung *et al*. 2006, Parham *et al*. 2006, Drosopoulou *et al*. 2012). The reconstruction of the evolutionary history of the Cheloniidae has been performed via phylogenetic tree analyses based on genetic data from the entire mitogenome (Kim *et al*. 2005, Duchêne *et al*. 2012, Drosopoulou *et al*. 2012).

In this study, the complete mitochondrial genome of a loggerhead turtle of the Colombian Atlantic Ocean (Cc-AO-C) was sequenced and analyzed with three purposes: (1) to compare the characteristics of mitochondrial genome with all previously reported mitogenomes of loggerhead individuals nesting in Hawaii, Pacific Ocean (Cc-PO-H); Peru, Pacific Ocean (Cc-PO-P); Greece, Mediterranean Sea (Cc-MS-G) and Florida, Atlantic Ocean (Cc-AO-F). (2) Assessing the mutations in the tRNAs genes and their possible implications in 2D and 3D structures, and (3) revisiting the phylogeny of the superfamily Chelonioidae using data from 13 protein-coding genes, the 16S rRNA gene, and the complete mitochondrial genome.

Materials and methods

Sampling, DNA extraction and quantification

A single blood sample was collected from a loggerhead turtle. This turtle was found at Don Diego beach (11°16' N - 73°45' W) in the Colombian Caribbean. This turtle showed the morphological characteristics of the logger head species Suppl. 1, was in good health condition, and had no evident physical anomalies. The sample was obtained from the dorsal cervical sinus of the turtle according to Dutton's method (1996). The blood sample was taken in a test tube with Tris-EDTA buffer to avoid coagulation and transported at 4 °C to the Molecular Biology Laboratory at the Universidad Jorge Tadeo Lozano, in Bogotá.

Total genomic DNA was extracted by using the GF-1 Tissue DNA Extraction Kit (Vivantis, Subang Jaya, Malaysia) according to manufacturer's instructions. The amount of DNA extracted was measured with a Nanodrop 1000 and analyzed with the ND-1000 V3.7.1 program (Thermo Scientific, Waltham, USA).

Primer design

The sequencing strategy for the entire mitogenome was based on PCR amplification of overlapping fragments of 800 - 2 500 bp in length. The overlap among fragments was of 50 - 200 bp to facilitate full sequence assembly. A total of 22 primer pairs were employed to sequence the mitogenome of the Colombian Caribbean loggerhead turtle (**Table 1**). Seventeen primer pairs were designed using the Overlapping Primer Sets Program (Whitehead Institute, Cambridge, USA) based on the mitochondrial genome sequence of another loggerhead sea turtle (Cc-MS-G, GenBank accession number NC_016923.1). The remaining five primer pairs were used as previously described by Drosopoulou *et al*. (2012).

Different analyses were performed to identify chimeras between the mitochondrial genome and nuclear paralog sequences. First, the mitochondrial DNA was assembled with the reference genome of the loggerhead turtle (GenBank accession number NC_016923.1). Then, the mitogenome was aligned with mitogenomes of other four loggerhead turtles, and a phylogenetic tree was built using the complete mitogenome sequences of all six sea turtle species.

Complete mtDNA amplification and sequencing

PCR reactions were carried out to a final volume of 25 μl. Each PCR reaction included: 1X PCR buffer (160 mM $(NH_4)_2SO_4$, 67 mM Tris-HCl [pH 8.8 to 25 °C], 0.1 % Tween-20), (Bioline Inc., Oxnard, USA), 1.5-3.0 mM de $MgCl_2$,

Table 1. Sequence, position and amplified genes of the loggerhead turtle Cc-AO-C mitogenome with primer pairs used as described in the text.

Oligo	5′-3′ Forward	3′-5′ Reverse	Gene	Position	Reference
CC1	GGCAGTAAAGTTCATTCGTTCCTC	GCCGATTGGTTGTTAGTTTGGG	D-Loop-, 12S	15925-358	Drosopoulou *et al.* 2012
CC2	GCCACCGCGGTTATACAAG	CAGTTAGCTACACCTTGACC	12S	315-788	Drosopoulou *et al.* 2012
CC3	ATCTACCTCACCATCCCTTGCC	GTCTTTCCACTCTTTTGCCACAG	12S, 16S	662-1441	Drosopoulou *et al.* 2012
CC4	CCTAAACAATTAATAAGTCA	TTAAGTACTTTATGTTGTTT	12s-16s	750-1550	Present study
CC5	TAGCTGGTTGCTCAATAAAA	TCGCCCCAACCAAAAATATAG	16s	1500-2300	Drosopoulou *et al.* 2012
CC6	CTGACTAAGCCCTAAAAAGCAAAG	CCCTGGGGTAGCTTGGTTCGTTGAT	16S	1269-2437	Present study
CC7	GGACTCCCACCTATACCATAG	TTGTAATTATTCATCCTAGATGGG	ND2	2250-3050	Present study
CC8	TACGAAAAATCATAGCATTC	ATTGCAAATTTAAAGATATATCT	ND2	3750-4550	Present study
CC9	TAAAAAGCGGGAAAACCCAG	GTTGTATTTAGATTTCGGTCTGT	COI	4500-5300	Present study
CC10	GTACTCGCCGCAGGCATTACCA	ATAACTACTGCTACTATAGAGA	COI	6000-6800	Present study
CC11	AACTCTATTTCATCAATTGG	TCAGCTGAGATTAGTATTC	COI-COII	6750-7550	Present study
CC12	ATCGCATAGTAATACCAATA	TCATATTGGAATAGCTAGTC	COII-ATP8-ATP6	7500-8300	Present study
CC13	CTACTAGGCCTTCTACCCTA	TGGGGTAATTCCTGTAGGTG	ATP6-COIII	8250-9050	Present study
CC14	CCCCTACCCCAGAACTAGGA	AAGGTGAGAGTTGGGGATGG	COIII-ND3	9000-9800	Present study
CC15	CGCCTGATACTGACACTTCG	CGTTTTCGTGAGGTTGGTTC	ND3-ND4L	8930-9830	Present study
CC16	AACCCATGGTTCAGACCAAC	GTGTAATTGCGCCGGTGTAG	ND4L-ND4	9730-10630	Present study
CC17	CACTGAAAACGGTTCCTCATC	GGGTGATTAGGGCTAAGAGG	ND4-ND5	10530-11430	Present study
CC18	ACACAATGAGGGGAAACACC	CTAGGCAAAGGCAGGTTGAG	ND5	11330-12230	Present study
CC19	CAGGAAAATCAGCCCAATTC	GATGTGTTGCGATGTTTTGG	ND5-ND6	12230-13030	Present study
CC20	GGAGTAATCCAGGTCGGTTTC	GGAATAGAATGGTGGTTAGGG	ND1	12930-13830	Present study
CC21	GCCTCAAACTCCAAATACGC	GTGGTAGGCCTGAGAAGGTG	ND1	13730-14730	Present study
CC22	TACCCCACGAGATAAACCACG	TAAGTATTCTGCACGGCCAATCA	ND6, CytB	13636-16135	Drosopoulou *et al.* 2012

0.4-1.0 μM of each forward and reverse primer, 200 μM of each dNTP, 1U of Taq Polymerase and 60 ng of DNA (Bioline Inc., Oxnard, USA). The employed thermocycling program consisted of an initial denaturation step at 94 °C for 5 min, followed by 35 cycles at 94 °C for 1 min, 30 °C - 50 °C (depending on each primer pair) for 1 min, 72 °C for 1 min, and a final extension step at 72 °C for 10 min. The PCR reaction was carried out in a block PTC-100TM Programmable Thermal Controller Thermocycler (MJ Research, Madison, USA). Complete standardization to this protocol was described by Beltran-Torres *et al* (2013).

The PCR-amplified electrophoretic bands were purified using the GF-1 Gel DNA Recovery kit (Vivantis Malaysia HQ). Purified material was used for double strand (5'-3' and 3'-5') sequencing reactions, using the automated tag DyeDeoxy Terminator Cycle-sequencing method in an ABI 3730XL sequencer (Applied Biosystems, Foster City, USA) at SSIGMOL, Universidad Nacional de Colombia, Bogotá, Colombia.

Mitogenome assembly

The 22 obtained sequences were aligned using ClustalW (Thompson *et al*. 1994) and assembled by means of the Geneious R6 program (Biomatters Ltd., Auckland, New Zealand) using the Cc-MS-G mitogenome as reference sequence (GenBank accession number NC_016923.1). To poceed with the assembly of the Cc-AO-C loggerhead sea turtle mtDNA reads to the reference sequence as FASTA files, the following options were used: File, import from file and finally Map to Reference. To determine the nucleotide composition of the assembled mitogenome, the "statistics" option was run. The Geneious Basic Local Alignment Search Tool (BLAST) (Altschul *et al*. 1990) was used to identify sequence similarities among the newly generated assembly and other loggerhead mitogenome assemblies previously mentioned. Functional annotation of the Cc-AO-C mitogenome assembly was made using BLASTX.

Genetic variation analysis

Standard diversity indices, such as number of haplotypes (k), number of polymorphic sites (S), haplotype diversity (H), average number of differences between pairs of sequences (II), and nucleotide diversity (π) according to Nei (1987) were estimated for each one of the thirteen mitochondrial protein coding genes from ad hoc sequence alignments of the Cc-AO-C turtle sequence (accession number KP256531.1) with sequences of other four loggerhead mitogenomes. These loggerhead mitogenomes were downloaded from the NCBI database and consisted of the Cc-AO-F (Florida-USA) (accession number JX454983), Cc-MS-G (Greece) (accession number NC_016923),

Cc-PO-P (Peru) (accession number JX454988), Cc-PO-H (Hawaii) (accession number JX454977). All genetic variation estimators were obtained with DNAsp v5.10 (Librado & Rozas, 2009). A similar approach was also applied to the D-Loop region of the Cc-AO-C and the afore mentioned four loggerhead mitogenomes and a set of 92 loggerhead D-Loop haplotype stretches of the Archi Carr Center for Sea Turtle Research at the University of Florida (accstr.ufl.edu).

tRNA structure analysis

Prediction of tRNA 2D structures were done with ARWEN (http://mbio-serv2.mbioekol.lu.se/ARWEN/) (Laslett & Canbäck 2008) and 3D structures were predicted with the 3D RNA composer Program (Popenda *et al*. 2012) (http://rnacomposer.cs.put.poznan.pl/Home/Compose). All structures were visualized using Geneious R6 in pdb format. The 3D structure of the tRNAs of Cc-AO-C was compared to those described for the loggerhead turtles of Cc-AO-F, Cc-MS-G, Cc-PO-P and Cc-PO-H. These tRNA data were also used to perform intraspecific analyzes.

Phylogenetic analysis

Phylogenetic inferences were made for the superfamily Chelonioidae using data from individual genes and complete mitochondrial genomes. The inference was made with mitogenome sequence data from seven sea turtle species: *C. mydas* (Cm-AO) (accession number NC_000886.1), *N. depressus* (Nd-PO-A) (accession number NC_018550.1), *E. imbricata* (Ei-AO-C) (accession number KP2218061), *C. caretta* (Cc-MS-G) (accession number NC_016923), *L. olivacea* (Lo-PO-CR) (accession number NC_028634.1), *L. kempi* (Lk-AO-US) (accession number JX_454982.1), and the mitogenome described in this study. The mitochondrial genome of *D. coriacea* (Dc-AO-US) (accession number JX_454989.1) was used as an outgroup.

Phylogenetic analyses were performed using three approaches, Maximum Likelihood (ML), Bayesian Inference algorithms (BI), and Maximum Parsimony (MP). ML and BI analyses were made with Geneious R6 (Biomatters Ltd., Auckland, New Zealand) and MP with MEGA 5.2. (Tamura *et al*. 2011). The models of nucleotide substitution of Tamura-Nei (TN), Hasegawa, Kishino and Yano (HKY), and the Generalized Time Reversible (GTR) model were used in the construction of phylogenetic trees. These models were chosen based on the lowest scores of the Bayesian Information Criterion (BIC) implemented in MEGA 5.2. (Nei & Kumar 2000, Tamura *et al*. 2011). For ML- and MP-based phylogenetic analyses, 1 000 bootstrap replicates were performed to generate a good statistical support.

Bootstrap values above 70 % were accepted as strong enough support for the different branches, according to Hillis & Bull (1993). 10 000 iterations were performed for the BI analysis. A consensus tree, with posterior probability values expressed in percentages, was obtained.

Results and discussion

The Cc-AO-C mitogenome sequence

The complete mitogenome sequence (16 362 bp in length) of the loggerhead turtle individual Cc-AO-C was obtained and deposited in the GenBank under accession number KP256531.1. Upon analysis of this mitogenome sequence, we confirmed that the sampled turtle was indeed a member of the *Caretta caretta* species. Since hybrids between sea turtles have been frequently reported (Bowen & Karl, 2007; Drosopoulou *et al*. 2012; Duchêne *et al*. 2012), it was necessary to ascertain that the captured mitogenome was indeed from the loggerhead species. In addition, attention was paid during primers design to avoid unintended amplification and sequencing of nuclear paralogs of some mitogenome genes. Moreover, obtained sequence reads were inspected for double peaks, as seen in diploid nuclear sequences, before mitogenome assembly.

As revealed by its base composition, the sampled mitogenome was A-rich (35 %) and had a low content of G (12.2 %), with intermediate C and T contents of 27 % and 25.7 %, respectively. The mitogenome of the Cc-AO-C turtle contains 13 protein-coding genes (ND1, ND2, ND3, ND4, ND4L, ND5, ND6, COI, COII, COIII, ATP6, ATP8, Cytb) two rRNA genes (12S and 16S), 22 tRNA genes and one non-coding control region (D-Loop).

Regarding protein-coding, rRNA, and tRNA genes, the obtained sequence of the Cc-AO-C mitogenome corresponded well to functional and codon usage annotations reported for other marine turtles (Kumazawa & Nishida 1999, Duchêne *et al*. 2012, Drosopoulou *et al*. 2012) (Suppl. 1)

Sequence variation across mitogenome protein-coding genes and D-Loop

The degree of sequence identity for all genes and functional units of the obtained loggerhead mitogenome was assessed against each of the other four loggerhead mitogenome sequences. Across all protein-coding genes, the average pair-wise sequence conservation degree was 98.49 % (**Table 2**).

Table 2. Identity percentages for all mitochondrial protein-coding genes between the Cc-AO-C and each mitogenome sequence of the four loggerhead mitogenomes: Cc-AO-F, Cc-MS-G, Cc-PO-P and Cc-PO-H.

Mitogenome	ATP8	ATP6	COI	COII	COIII	ND1	ND2	ND3	ND4	ND4L	ND5	ND6	CyTb	D-Loop
	Cc-AO-C													
Cc-AO-F	100	100	100	94.2	99.0	99.7	100	98.3	99.8	99.7	97.6	99.6	99.7	94.4
Cc-MS-G	100	100	99.9	94.2	99.0	99.8	100	98.3	99.8	100	97.6	99.6	99.7	94.6
Cc-PO-P	99.4	99.6	99.4	93.5	98.7	98.6	99.2	96.0	99.7	98.3	97.0	99.0	99.5	96.9
Cc-PO-H	90.0	99.6	99.4	93.5	98.7	98.6	98.8	96.0	99.7	98.3	97.0	99.0	99.5	96.1

The lowest level of sequence similarity between the Cc-AO-C mitogenome and the set of four loggerhead turtle mitogenome sequences was observed at the COII gene (93-94 %); and across functional units, the Cc-AO-C mitogenome was most similar with the mitogenomes of the Cc-PO-F and Cc-PO-G turtles. Furthermore, it is interesting to see how the levels of genetic identity between the mitochondrial genes of Cc-AO-C and these Atlantic-Mediterranean turtles are higher than those between the mitochondrial genes of Cc-AO-C and the turtles of the Pacific Ocean. This observation can substantiate the possibility of genetic flow between Atlantic-Mediterranean turtles, which is further supported by various studies on their broad migratory routes and geographical range. For instance, Casale *et al* (2013) provided the first evidence of a migratory connection of a loggerhead from the Mediterranean to North America. Besides, there is evidence that loggerhead turtles born on northwestern Atlantic beaches disperse as far as eastern Atlantic coasts, and some of them even enter the Mediterranean Sea (Bolten 2003) where they share foraging grounds with the Mediterranean population (Monzon-Arguello *et al*. 2010, Wallace *et al*. 2010, Carreras *et al*. 2011).

Sequence analysis of the ND3 gene of the Cc-AO-C mitogenome reveladed an A insertion at position 175. This programed frameshift mutation has only been seen before in the Cc-MS-G mitogenome (Drosopoulou *et al*. 2012), and it is a likely neutral variant since it does not lead to protein sequence changes. Moreover, the same mutation has been described in other turtle species, reptiles and birds, and it is considered as relatively ancestral (Russel & Beckenbanch 2008).

The parts of the mitogenome that showed the highest average number of differences between pairs of sequences (Π), and thus the greatest genetic variation (**Table 3**) and the lowest sequence identity values (Table 2) were the D-Loop and protein-coding genes COII and ND5. Compared to other mitochondrial functional units, the D-Loop has been reported as the stretch with the highest levels of genetic diversity among sea turtle populations (Abreu-Grobois *et al*, 2006; Novelletto *et al*. 2016) as a non-coding and likely neutrally evolving DNA stretch, the D-Loop is possibly one of the top informative mitogenome fragments to perform gene flow analyses in populations of the species *C. caretta*. Based on the current results, the genes COII and ND5 could be equally useful when employed for this type of analyses.

In contrast, the gene ATP8 was devoid of any sequence variation in the studied sequence set, thus having the highest degree of conservation. The availability of sequence data for the D-Loop from a broader sample of loggerhead turtles, allowed further investigation on D-Loop haplotype sequence identity across specific geographic ranges. The D-Loop haplotype of the Cc-AO-C mitogenome was most identical (99.2 %) with the CC-A1 haplotype, which is the most frequent (> 80 %) in nesting colonies along the North American east coast (North Carolina to South Florida) The CC-A1 haplotype has also been found in loggerhead turtles in the Colombian Caribbean (Franco & Hernandez, 2012, 2017). A total of 13 sites account for the differences between the Cc-AO-C and CC-A1 haplotype sequences. Thus, the Cc-AO-C haplotype can be regarded as novel among those described for nesting loggerhead turtles in the Caribbean.

The haplotype that showed 95 % identity with Cc-AO-C D-Loop was CC-A2. This haplotype has also been reported in the Colombian Caribbean (Franco & Hernandez, 2012, 2017), and it is the dominant haplotype in loggerhead turtles of samples in Quintana Roo (Mexico), southwestern Cuba (Ruiz-Urquiola *et al*. 2010), and the South Florida rookeries (SE and SW combined).

Prior studies have reported opposite latitudinal gradients in the frequencies of these two main haplotypes in the Caribbean. The CC-A2 haplotype is most frequent in the north and becomes less common southward, whereas the opposite pattern is seen for haplotype CC-A1 (Encalada *et al*. 1998; Bowen *et al*. 2005 and Shamblin *et al*. 2011). Nesting aggregation in Colombia is related to nesting colonies in Southern Florida and Mexico. Loggerhead turtles from the foraging area around Don Diego beach (in the Colombian Caribbean) 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

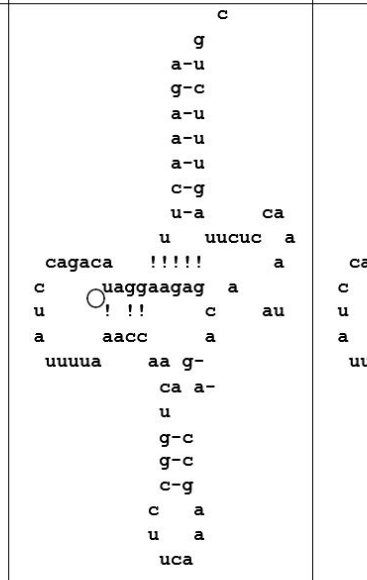
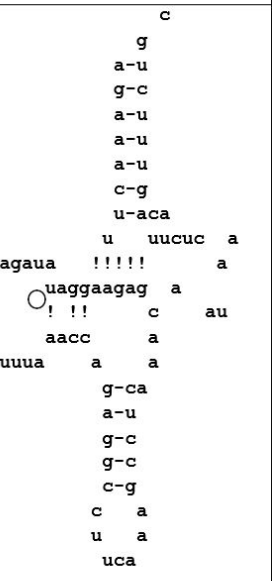
Table 3. Genetic diversity estimators, for each mitochondrial proteincoding gene and the D-Loop region, for a loggerhead alignment consisting of the Cc-AO-C sequence and four loggerhead mitogenomes: Cc-AO-F, Cc-MS-G, Cc-PO-P and Cc-PO-H (see text for sequence name details).

	Gene/ Region	Fragment length (bp)	Number of polymorphic sites (S)	Number of Haplotypes (k)	Haplotypic diversity (H)	Nucleotide Diversity (π)	Average number of pair-wise differences (Π)
A	ATP6	684	3	2	0.600 ± 0.175	0.02640	1.800
B	ATP8	165	-	1	0.000 ± 0.000	0.00000	0.000
C	COI	1548	10	3	0.800 ± 0.164	0.00375	5.800
D	COII	687	38	3	0.800 ± 0.164	0.02438	16.600
E	COIII	784	11	3	0.800 ± 0.164	0.00663	5.200
F	Cytb	1145	8	3	0.800 ± 0.164	0.00385	4.400
G	ND1	978	14	3	0.800 ± 0.164	0.00807	7.800
H	ND2	1042	7	2	0.600 ± 0.175	0.00405	4.200
I	ND3	352	14	3	0.800 ± 0.164	0.02057	7.200
J	ND4	1381	7	3	0.800 ± 0.164	0.00304	4.200
K	ND4L	297	5	3	0.800 ± 0.164	0.01010	3.000
L	ND5	1797	56	3	0.800 ± 0.164	0.01436	25.000
M	ND6	525	6	3	0.800 ± 0.164	0.00571	3.000
J	D-Loop	681	36	3	0.800 ± 0.026	0.02891	19.400

Atlantic and Mexico. This pattern suggests that individuals that use the Colombian Caribbean for feeding and reproduction are part of an Atlantic meta-population, where haplotypes CC-A1 and CC-A2 are the most frequent (Franco & Hernandez, 2012, 2017). The novel Cc-AO-C loggerhead haplotype may be endemic to the Colombian Caribbean rookery, and thus may suggest that Colombian loggerheads display natal homing.

tRNA variation

The 22 tRNAs were distributed along the mitogenome (13 in the H-strand and 9 in the L-strand). When the 22 tRNAs sequences of the Cc-AO-C and Cc-MS-G turtles were compared, 9.1 % (2 out of 22, $tRNA^{Trp}$ and $tRNA^{Leu}$) revealed nucleotide differences (**Fig. 1**). The remainder 90.9 % (20) revealed a strong nucleotide conservation. Such level of conservation may be due to

tRNA/Typology	Mediterranean *Caretta caretta*	Colombian Caribbean *Caretta caretta*
$tRNA^{Trp}$ (Triptófano): Transition Pyrimidine to pyrimidine. Cytosine to uracil **Typology II** (Suzuki et al. 2011)		

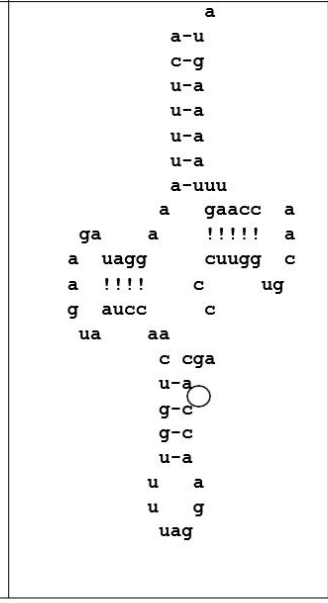
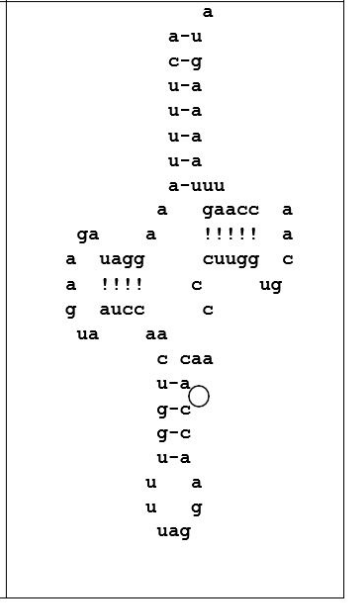
tRNA/Typology	Mediterranean *Caretta caretta*	Colombian Caribbean *Caretta caretta*
$tRNA^{Leu}$ (Leucina CUN): Transición purine te purina. Guanine to adenine. **Typology II** (Suzuki et al. 2011)		

Figure 1. Comparison of 2D structure of $tRNA^{Trp}$ and $tRNA^{Leu}$ between both, Cc-AO-C and Cc-MS-G that presented mutations (marked with circles).

the small size of these tRNAs and the selective pressure exerted on these important elements for the process of molecular translation (Florentz *et al*. 2003, Widmann *et al*. 2010) (Fig. 1).

Secondary typology analysis of the Cc-AO-C tRNAs revealed unique features at $tRNA^{Trp}$ and $tRNA^{Leu}$. These two tRNAs present typology II as defined by Suzuki *et al* (2011) (Fig. 1). The unique features at Cc-AO-C $tRNA^{Trp}$ and $tRNA^{Leu}$, however, did not lead to large changes in the predicted 3D structure of these tRNAs, with respect to other loggerhead structures of Cc-PO-H, Cc-PO-P, Cc-MS-G, and Cc-AO-F (**Fig. 2**). The $tRNA^{Trp}$ was characterized by presenting tertiary interactions among positions 16 - 48, at the tRNA's D-loop allowing the folding of the structure (Saks *et al*. 1998, Suzuki *et al*. 2011). Furthermore, this Cc-AO-C $tRNA^{Trp}$ presented a transition in position 14 (Fig. 1) which does not allow for any interaction with position 48, leading to a modified 3D structure (Fig. 2). These structural changes might have a negative impact on the individual, and they should be studied at the population level to determine their actual frequency. Moreover, it is essential to verify if there are heteroplasmic mutations, and finally, study whether those changes can lead to a pathologic state of the turtles.

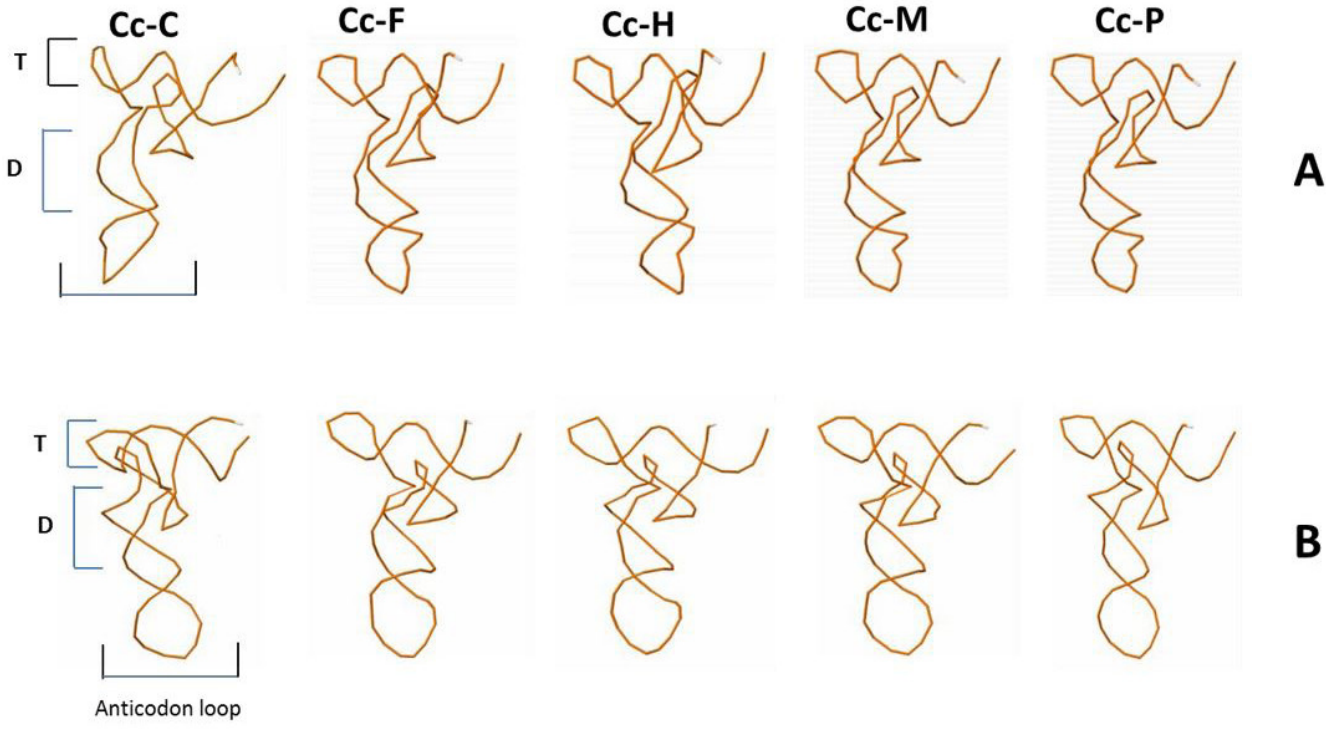


Figure 2. 3D tRNA structures of the Cc-AO-C turtle with respect to the Cc-AO-F, Cc-PO-H, Cc-PO-P, and Cc-MS-G loggerhead turtle tRNA structures. A. $tRNA^{Trp}$ B. $tRNA^{Leu}$. Regions of the tRNAs: (D) D-Loop, (T) T-loop, (A) anticodon are shown.

Phylogenetic inference of marine turtles

The individual markers that best explained the phylogeny of the sea turtles were ND5, ND4, and 16S when using the BI method. The ND5 gene has not been yet used as a molecular marker to do phylogenetic analysis in sea turtles (**Fig. 3**). However, in the present study the topology obtained with this gene is in full agreement with the currently accepted sea turtle phylogeny. These results support the analysis done by Dutton *et al* (1996) who used ND5 gene data to lay out a phylogenetic hypothesis for these organisms.

Cases of phylogenetic incongruity among individual genes were found. For instance, trees based on data from the ATP8 and ND4L genes were not informative (results not shown), likely due to their small size and high level of nucleotide conservation (Table 2 and 3). These two genes are essential part of enzyme production in the mitochondria (Suzuki *et al*. 2011). The gene

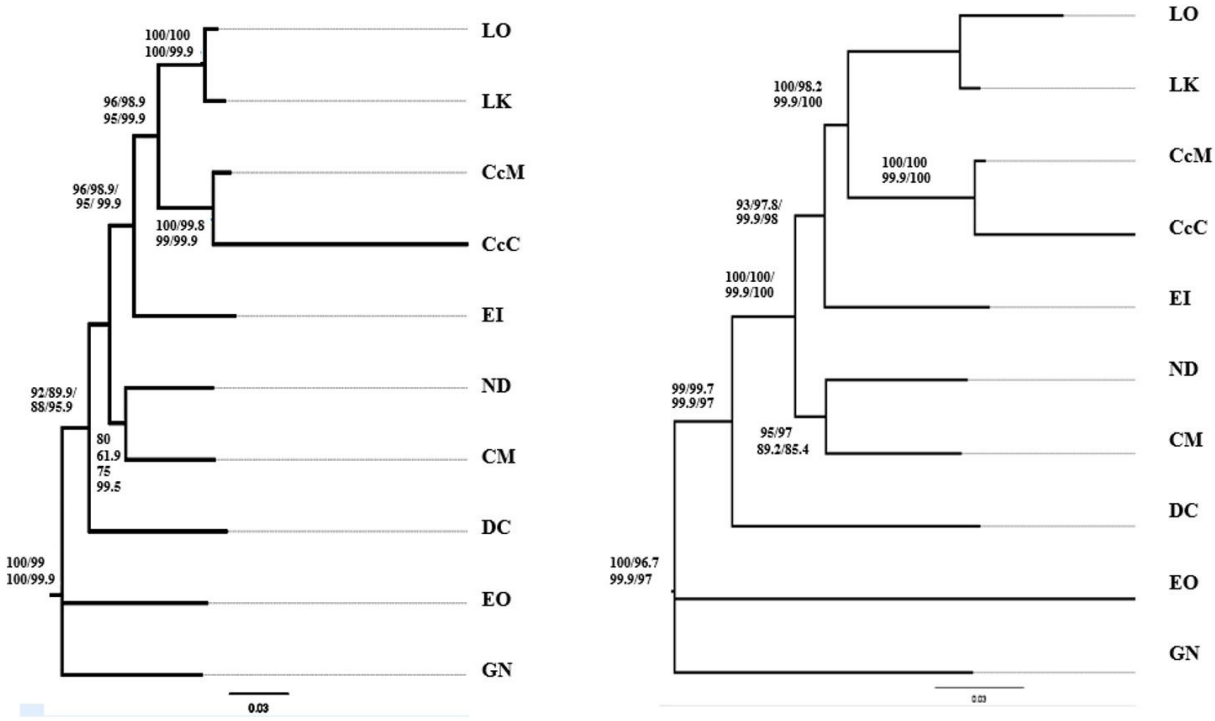


Figure 3. Phylogenetic inference of sea turtles. A: Tree based on data from gene ND4 employing Maximum Likelihood (ML), Maximum Parsomony (MP), and Bayesian Inference (BI) methods. B. Tree from complete mitogenome data following ML, MP, and BI methods. Following samples were included: *L. kempii* (LK), *L. olivacea* (LO), *C. caretta*- Colombian Atlantic Ocean (Cc-AO-C), *C. mydas* (CM), *C. caretta*- Greek Mediterranean Sea (Cc-MS-G), *N. depressus* (ND), *E. imbricata* (EI), and *D. coriacea* (DC).

ND1 resolved relations within the Cheloniidae family but was not useful in differentiating Cheloniidae from Dermochelyidae. Previous molecular studies have not established a coherent and statistically well-supported conclusion on the phylogeny of sea turtles (Kumazawa & Nishida 1999).

In current phylogenetic analysis, the use of data from complete mitogenomes is gaining ground. With full or partial mitogenome data, phylogenetic analyses become more robust and gain in phylogenetic resolution and greater precision compared to analysis based on data from individual markers (Duchêne *et al*. 2011). The current results support previous relationships among sea turtle species, *N. depressus* as the sister taxon to Chelonia (Duchêne *et al*. 2012, Naro-Maciel *et al*. 2008) as well as the clade comprising *Erecmochelys, Lepidochelys* and *Caretta* (Fig. 3) (Dutton *et al*. 1999, Duchêne *et al*. 2012). This result is important when explaining phylogenetic relationships within the family Cheloniidae, particularly the exclusion of *N. depressus* from the subfamily Carettini (Dutton *et al*. 1999, Duchêne *et al*. 2012, Naro-Maciel *et al*. 2008). Out of the total number of mitochondrial markers, from which data were obtained to solve ancestry-descent relations among sea turtles, the ND5 gene produced highly supported trees. This marker can generate phylogenetic trees with a support comparable to that of a complete mitochondrial genome, and it confirms the topology of the proposed phylogeny for these species. This study presents the use of mitochondrial genomes as an alternative to improve phylogenetic analysis to estimate the evolutionary relations among sea turtles.

Conclusions

In this study, the complete mitochondrial genome of an individual of the endangered loggerhead marine turtle species, *C. caretta*, nesting in the Colombia Caribbean coast was sequenced. This has opened new possibilities to understand the extent of genetic variation and how matrilineal gene flow happens within the loggerhead species across its broad distribution range.

This loggerhead mitogenome is 16 362 bp long, comprises a non-coding region (D-Loop), 13 protein-coding genes, 22 tRNA genes and 2 rRNA (16S and 12S). This sampled nesting turtle harbors a new D-Loop haplotype, with thirteen sites differing from the closest previously reported Caribbean CC-A1 haplotype. The $tRNA^{Trp}$ and $tRNA^{Leu}$ presented specific mutations in Cc-AO-C. The other 20 tRNAs revealed a strong nucleotide conservation and $tRNA^{Trp}$ presented modification of its 3D structure.

The phylogeny of sea turtles was revisited with this novel mitogenome. The entire mitogenome, and the loci ND5, ND4, and 16S provided sequence data to build well resolved trees that largely agreed with currently accepted

sea turtle phylogenetic hypotheses. This study presents the use of complete mitogenomes as a feasible alternative to gather data useful to conduct thorough phylogenetic analysis in sea turtles.

Acknowledgements

The authors would like to thank Jaime Rojas at the Ceiner Oceanarium for the loggerhead turtle blood sample collection and to the anonymous reviewers who have contributed their time and expertise to improve this manuscript. Financial support for this study was granted by the Research, Creativity, and Innovation Department at Universidad Jorge Tadeo Lozano.

Research Permissions

The samples were obtained under research permission granted by the Ministerio del Medio Ambiente y Desarrollo Territorial (No 24, June 22, 2012) and the Access Contract to Genetic Resources (No 64, April 23, 2013).

Conflicts of interests

The authors declare no conflict of interest and state that they are responsible for content and writing of the paper.

References

Archi Carr Center for Sea Turtle Research (ACCSTR)

http://accstr.ufl.edu/. Acces in May 20 of 2018

Abreu-Grobois FA, Horrocks J, Formia A, Dutton PH, LeRoux R, Vélez-Zuazo X, Meylan P. New mtDNA Dloop primers which work for a variety of marine turtle species may increase the resolution of mixed stock analyses, In *Proceedings of the 26th annual symposium on sea turtle biology*. Island of Crete, Greece: ISTS. p. 179, 2006.

Adebambo AO. Mitochondrial DNA D-Loop analysis of south western Nigeria. Department of Animal Breeding and Genetics. University of Agriculture. Abeokuta. Archives, *Archive of Zootecnia*, 58: 637-643, 2009.

Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ. Basic local alignment search tool, *Journal of Molecular Biology*, 5, 215(3): 403-10, 1990.

doi: 10.1016/S0022-2836(05)80360-2

Amorocho D. Monitoring nesting loggerhead turtles (*Caretta caretta*) in the central Caribbean coast of Colombia, *Marine Turtle Newsletter*, 101: 8-13. 2003

Avise JC. Molecular Markers, natural history and evolution. London: Chapman and Hall. 1994.

Beltrán G, Acevedo KO, Daza LA, Hernández J. Estandarización de la técnica de PCR para amplificar el genoma mitocondrial de las tortugas cabezona (*Caretta caretta*) y carey (*Eretmochelys imbricata*) anidantes del Caribe colombiano, *Mutis*, 3(2): 21-30, 2013.

doi: 10.21789/22561498.882

Bolten AB. Variation in sea turtle life history patterns: neritic vs. oceanic developmental stages, *The biology of sea turtles*, 2, 243-257, 2003.

doi: 10.1111/j.1365-294X.2005.02598.x

Bowen BW, Karl SA. Population genetics and phylogeographyof sea turtles, *Molecular Ecology*, 16: 4886-4907, 2007.

doi: 10.1111/j.1365-294X.2007.03542.x

Bowen BW, Abreu-Grobois FA, Balazs GH, Kamezaki N, Limpus CJ, Ferl RJ. Trans-Pacific migrations of the loggerhead turtle (*Caretta caretta*) demonstrated with mitochondrial DNA markers, *Proceedings of the National Academy of Sciences*, 92 (9) 3731-3734, 1995.

doi: 10.1073/pnas.92.9.3731

Bowen BW, Bass AL, Soares L, Toonen RJ. Conservation implications of complex population structure: lessons from the loggerhead turtle (*Caretta caretta*), *Molecular Ecology*, 14(8), 2389-2402, 2005.

doi: 10.1111/j.1365-294X.2005.02598.x

Carreras C, Pascual M, Cardona L, Marco A, Bellido JJ, Castillo JJ, Tomas J, Raga JA, Sanfelix M, Fernandez G, Aguilar A. Living together but remaining apart: Atlantic and Med-iterranean loggerhead sea turtles (*Caretta caretta*) in shared feeding grounds, *The Journal of Heredity*, 102: 666-677, 2011.

doi: 10.1093/jhered/esr089

Casale P, Freggi D, Dourdeville KM, Prescot R. First evidence of migration by loggerhead sea turtles, *Caretta caretta*, from the eastern Mediterranean to North America, *Vie et Milieu*, 63(2): 93-96, 2013.

Ceballos-Fonseca C. Distribución de playas de anidación y áreas de alimentación de tortugas marinas y sus amenazas en el Caribe colombiano, *Boletín de Investigaciones Marinas y Costeras*, 33: 79-99, 2004.

Chiari Y, Cahais V, Galtier N, Delsuc F. Phylogenomic analyses support the position of turtles as the sister group of birds and crocodiles (Archosauria), *BMC Biology*, 10(1): 65-68, 2012.

doi: 10.1186/1741-7007-10-65

Dodd CK. Synopsis of the biological data on the loggerhead sea turtle *Caretta caretta* (Linnaeus 1758). Fish and Wildlife Service, *Biological Report*, 88(14): 1-110, 1988.

Drosopoulou E, Tsiamis G, Mavropoulou M, Vittas S, Katselidis KA, Schofield G, Palaiologou D, Sartsidis T, Bourtzis K, Pantis J, Scouras ZG. The complete mitochondrial genome of the loggerhead turtle *Caretta caretta* (Testudines: Cheloniidae): Genome description and phylogenetic considerations, *Mitochondrial DNA*, 23(1): 1-12, 2012.

doi: 10.3109/19401736.2011.637109

Duchêne S, Archer FI, Vilstrup J, Caballero S, Morin PA. Mitogenome phylogenetics: the impact of using single regions and partitioning schemes on topology, substitution rate and divergence time estimation, *PloS One*, 6(11): e27138, 2011.

doi: 10.1371/journal.pone.0027138

Duchêne S, Frey A, Alfaro-Núñez A, Dutton PH, Thomas P, Gilbert M, Morin PA. Marine turtle mitogenome phylogenetics and evolution, *Molecular Phylogenetics and Evolution*, 65(1): 241-250, 2012.

doi: 10.1016/j.ympev.2012.06.010

Dutton PH, Davis SK, Guerra T, Owens D. Molecular phylogeny for marine turtles based on sequences of the ND4- leucine tRNA and control regions of mitochondrial DNA, *Molecular Phylogenetic Evolution*, 5: 511-521, 1996.

doi: 10.1006/mpev.1996.0046

Dutton PH, Bowen BW, Owens DW, Barragan A, Davis SK. Global phylogeography of the leatherback turtle (Dermochelys coriacea), *Journal of Zoology*, 248(3), 397-409, 1999.

Eckert KL, Bjorndal KA, Abreu-Grobois, FA, Donnelly, M. Técnicas de Investigación y Manejo para la Conservación de las Tortugas Marinas. Grupo especialista en Tortugas Marinas. Unión Internacional para la Conservación de la Naturaleza y Comisión de Supervivencia de Especies, Publicación, (4), 2000.

Encalada S, Bjorndal K, Bolten A, Zurita J, Schroeder B, Possardt E, Sears C, Bowen B. Population structure of loggerhead turtle (*Caretta caretta*) nesting colonies in the Atlantic and Mediterranean as inferred from mitochondrial DNA control region sequences, *Marine Biology*, 130: 567-575, 1998.

Florentz C, Sohm B, Tryoen Toth P, Pütz J, Sissler M. Human mitochondrial tRNAs in health and disease, *Cellular and Molecular Life Sciences CMLS*, 60(7): 1356-1375, 2003.

doi: 10.1007/s00018-003-2343-1

Francisco AM, Bass AL, Bowen BW. Genetic characterization of loggerhead turtles (*Caretta caretta*) nesting in Volusia County. Department of Fisheries and Aquatic Sciences, University of Florida, Gainesville, 1999.

http://www.floridaconservation.org

Franco C, Hernández-Fernández J. Análisis de haplotipos de la tortuga cabezona *Caretta caretta* Testudines: Cheloniidae) en dos playas del Caribe colombiano, *Revista Lasallista Investigación*, 14(2): 121-131, 2017.

doi: 10.22507/rli.v14n2a11

Franco C, Hernández-Fernández J. Description of mtDNA Markers of Loggerhead Turtles from Caribbean Colombia, *Marine Turtle Newsletter*, 132: 3-4, 2012.

FitzSimmons N, Moritz C, Limpus C, Miller J, Parmenter C, Prince R. Comparative genetic structure of green, loggerhead, and flatback populations in Australia based on variable mtDNA and nDNA regions. 2-25. Proceedings of the International Symposium on Sea Turtle Conservation Genetics. NOAA Technical memorandum NMFS-SEFSC-396, Washington DC. p. 173. 1996.

FWC. A statistical analysis of trends in Florida's loggerhead nest counts with data through 2012. Florida Fish and Wildlife Conservation Commission. 2015

http://myfwc.com/research/wildlife/sea-turtles/nesting/loggerhead-trends/

Hatase H, Kinoshita M, Bando T, Kamezaki N, Sato K. Matsuzawa Y, Goto k, Omuta K, Nakashima Y, Takeshita H, Sakamoto W. Population structure of loggerhead turtles, *Caretta caretta*, nesting in Japan: bottlenecks on the Pacific population, *Marine Biology*, 141: 299-305, 2002.

Hernández-Fernández J, Pinzón A, Mariño-Ramírez L. De novo transcriptome assembly of loggerhead sea turtle nesting of the Colombian Caribbean, *Genomics Data*, 13: 18-20, 2017

doi: 10.1016/j.gdata.2017.06.005

Hillis DM, Bull JJ. An empirical test of bootstrapping as a method for assessing confidence in phylogenetic analysis, *Systematic biology*, 42(2): 182-19, 1993.

doi: 10.1093/sysbio/42.2.182

Humber F, Godley BJ, Broderick AC. So excellent a fishe: a global overview of legal marine turtle fisheries, *Diversity and Distributions*, 20: 579-590, 2014

doi: 10.1111/ddi.12183

IUCN. IUCN Red List of Threatened Species. Versión 2015.4.

www.iucnredlist.org. 2016

Jung So, Lee YM, Kartavtsev Y, Park IS, Kim DS, Lee JS. The complete mitochondrial genome of the Korean soft-shelled turtle *Pelodiscus sinensis* (Testudines, Trionychidae), *DNA Sequence*, 17: 471-483, 2006.

doi: 10.1080/10425170600760091

Kim IC, Jung SO, Lee YM, Lee CJ, Park JK, Lee JS. The complete mitochondrial genome of the rayfish *Raja porosa* (Chondrichthyes Rajidae), *DNA Sequence*, 16: 187-194, 2005.

doi: 10.1080/10425170500087975

Kumazawa Y, Nishida M. Complete mitochondrial DNA Sequences of the green turtle and blue-tailed mole skink: Statistical evidence for Archosaurian affinity of turtles, *Molecular Biology and Evolution*, 16: 784-792, 1999.

Lancheros-Piliego D, Hernández Fernández J. AMDAR and PCR-Extra-fast for Molecular identification of the loggerhead sea turtle *Caretta caretta* (Testudines: Cheloniidae) using the mitochondrial gene cytochrome c oxidase I (COI), *Universitas Scientiarum*, 18(3): 321-330, 2013.

doi: 10.11144/Javeriana.SC18-3.apit

Laslett D, Canbäck B. ARWEN, a program to detect tRNA genes in metazoan mitochondrial nucleotide sequences, *Bioinformatics*, 24: 172-175, 2008.

doi: 10.1093/bioinformatics/btm573

Librado P, Rozas J. DnaSP v5: a software for comprehensive analysis of DNA polymorphism data, *Bioinformatics*, 25: 1451-1452, 2009.

Machado A, Bermejo JA. Estado de conservación de la tortuga boba (*Caretta caretta*) en las islas Canarias. Plan de seguimiento de la tortuga boba en Canarias. Santa Cruz de Tenerife: Observatorio Ambiental Granadilla; 154p, 2012.

Marck C, Grosjean H. tRNomics: analysis of tRNA genes from 50 genomes of Eukarya, Archaea, and Bacteria reveals anticodon-sparing strategies and domain-specific features, *RNA*, 8(10): 1189-1232, 2002.

MITOMAP. A Human Mitochondrial Genome Database, 2018.

http://www.mitomap.org

Miya M, Takeshima H, Endo H, Ishiguro NB, Inoue JG, Mukai T, Satoh TP, Yamaguchi M, Kawaguchi A, Mabuchi K, Shirai SM, Nishida M. Major patterns of higher teleostean phylogePengs: A new perspective based on 100 complete mitochondrial DNA sequences, *Molecular Phylogenetics and Evolution*, 26: 121-138, 2003.

doi: 10.1016/S1055-7903(02)00332-9

Monzón-Arguello C, Rico C, Naro-Maciel E, varo-Cruz N, López P, Marco A, López-Jurado LF. Population structure and conservation implications for the loggerhead sea turtle of the Cape verde Islands, *Conservation Genetics*, 11(5): 1871-1884, 2010.

Naro Maciel E, Le M, Fitzsimmons N, Amato G. Evolutionary relationships of marine turtles: A molecular phylogeny based on nuclear and mitochondrial genes, *Molecular Phylogenetics and Evolution*, 49(2): 659-662, 2008.

doi: 10.1016/j.ympev.2008.08.004

Nei M, Kumar S. Molecular evolution and phylogenetics. Oxford University press. New York, 2000.

Novelletto A, Testa L, Iacovelli F, Blasi P, Garofalo L, Mingozzi T, Falconi M. Polymorphism in Mitochondrial Coding Regions of Mediterranean Loggerhead Turtles: Evolutionary Relevance and Structural Effects, *Physiological and Biochemical Zoology*, 89(6): 473-486, 2016.

doi: 10.1086/688679

Nobetsu T, Minami H, Matsunaga H, Kiyota M, Yokota K, Kimura N, Nakano H. Nesting and post-nesting studies of loggerhead turtles (*Caretta caretta*) at Omaezaki, Japan. National Research Institute of Far Seas Fisheries, Fisheries Research Agency. p. 30-33. 2004.

Parham JF, Feldman CR. Boore JL. The complete mitochondrial genome of the enigmatic bigheaded turtle (*Platysternon*): Description of unusual genomic features and the reconciliation of phylogenetic hypotheses based on mitocondrial and nuclear DNA, *BMC Evolution Biology*, 6: 11-21, 2006.

doi: 10.1186/1471-2148-6-11

Popenda M, Szachniuk M, Antczak M, Purzycka KJ, Lukasiak P, Bartol N, Adamiak RW. Automated 3D structure composition for large RNAs, *Nucleic Acids Research*, 40(14): e112, 2012.

doi: 10.1093/nar/gks339

Pritchard PCH, Mortimer JA. Taxonomy External Morphology, and Species Identification, p-21- 38. In: Eckert KL, Bjornald A. Abreu-Grobois and Donelly M. (eds), Research and Management Techniques for the Conservation of Sea Turtles. IUCN/SSC. Marine Turtle Specialist Group Public. No.4. Washington. D.C. 1999.

ReisLEC, Soares S, Vargas M, Santos FR, Young RJ, Bjorndal KA, Bolten AB, Lôbo-Hajdu G. Genetic composition, population structure and phylogeography of the loggerhead sea turtle: colonization hypothesis for the Brazilian rookeries, *Conservation Genetics*, 11(4): 1467-1477, 2010.

doi: 10.1007/s10592-009-9975-0

Ruiz-Urquiola A, Riverón-Giró F, Pérez-Bermúdez E, Abreu-Grobois F, González-Pumariega M, James-Petric B, Díaz-Fernández R, Álvarez-Castro J, Jager M, Azanza-Ricardo J, Espinosa-López G. Population genetic structure of Greater Caribbean green turtles (*Chelonia mydas*) based on mitochondrial DNA sequences, with an emphasis on rookeries from Southwestern Cuba, *Revista de Investigaciones Marinas*, 31 (1): 33-52, 2010.

Russell RD, Beckenbach AT. Recoding of translation in turtle mitochondrial genomes: programmed frameshift mutations and evidence of a modified genetic code, *Journal of Molecular Evolution*, 67(6): 682-695, 2008.

doi: 10.1007/s00239-008-9179-0

Saks VA, Veksler VI, Kuznetsov AV, Kay L, Sikk P, Tiivel T, Tranqui L, Olivares J, Winkler K, Wiedemann F, Kunz WS. Permeabilized cell and skinned fiber techniques in studies of mitochondrial function in vivo. In Bioenergetics of the Cell: Quantitative Aspects pp. 81-100. Springer US. 1998.

Shamblin BM, Dodd Mg, Bagley DN, Ehrhart LM, Tucker AD, Johnson C, Carthy RR, Scarpino RA, McMichael E, Addison DS, Williams KL, Frick MG, Ouellette S, Meylan AB, Godfrey MH, Murphy SR, Nairn CJ. Genetic structure of the southeastern United States loggerhead turtle nesting aggregation: evidence of additional structure within the peninsular Florida recovery unit, *Marine Biology*, 158: 571–587, 2011.

doi: 10.1007/s00227-010-1582-6

Scotto C. Análisis filogenético comparativo entre secuencias codificadoras (Cytb y ATPasa8) y secuencias no codificadoras (D-Loop) del ADN mitocondrial de primates y sus implicancias evolutivas en los homínidos, *Horizonte Médico*, 6(2): 111-129, 2006.

Suzuki T, Nagao A, Suzuki T. Human Mitochondrial tRNAs: Biogenesis, Function, Structural Aspects, and Diseases, *Annual Review of Genetics*, 45: 299-329, 2011.

doi: 10.1146/annurev-genet-110410-132531

SWOT. The state of the world's Sea turtles. The world's most (and least) threatened sea turtles. SWOT Report VII. Editorial Team. Arlington, USA. 2011

Retrieved: http://seaturtlestatus.org/sites/swot/files/report/030612_SWOT7_FinalA.pdf

Tamura K, Peterson D, Peterson N, Stecher G, Nei M, Kumar S. MEGA5: Molecular Evolutionary Genetics Analysis Using Maximum Likelihood, Evolutionary Distance, and Maximum Parsimony Methods, *Molecular Biology and Evolution*, 28(10): 2731-2739, 2011.

doi: 10.1093/molbev/msr121

Thompson JD, Higgins DG, Gibson TJ. CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice, *Nucleic acids research*, 22(22): 4673-4680, 1994.

doi: 10.1093/nar/22.22.4673

Wallace BP, DiMatteo AD, Hurley BJ, Finkbeiner EM, Bolten AB, Chaloupka MY, Hutchinson BJ, Abreu-Grobois FA, Amorocho D, Bjorndal kA, Bourjea J, Bowen BW, Duenas RB, Casale P, Choudhury BC, Costa A, Dutton PH, Fallabrino A, Girard A, Girondot M, Godfrey MH, Hamann M, Lopez-Mendilaharsu M, Marcovaldi MA, Mortimer JA, Musick JA, Nel R, Pilcher NJ, Seminoff JA, Troeng S, Witherington B, Mast RB. Regional Management Units for Marine Turtles: A Novel Framework for Prioritizing Conservation and Research across Multiple Scales, *PloS One*, 5(12): e15465, 2010.

doi: 10.1371/journal.pone.0015465

Widmann J, Harris K, Lozupone C, Wolfson A, Knight R. Stable tRNA-based phylogenies using only 76 nucleotides, *RNA*, 16: 1469-1477, 2010.

doi: 10.1261/rna.726010

Genoma mitocondrial completo de la tortuga caguama del Caribe colombiano: análisis de la estructura del tRNA y revisión de la filogenia de las tortugas marinas

Resumen. La tortuga marina caguama, *Caretta caretta*, es una especie ampliamente distribuida pero que enfrenta una crítica reducción de su población en las colonias del Caribe colombiano. Los datos de las secuencias de DNA mitocondrial son de gran importancia para la descripción, monitoreo y análisis de la filogenia de las tortugas migratorias. En este estudio se secuenció y analizó por primera vez el genoma mitocondrial completo de la tortuga caguama que anida en el Caribe colombiano. Este genoma tiene un tamaño de 16.362 pb con una composición de nucleótidos de T: 25.7 %, C: 27 %, A: 35 % y G: 12 %. La anotación de la secuencia de la molécula reveló una organización y número de unidades codificantes y funcionales como los reportados para mitogenomas de otros vertebrados. Esta tortuga caguama colombiana (Cc-AO-C) mostró un nuevo haplotipo D-Loop que contiene trece nuevos sitios variables, que comparten el 99.2 % de identidad de secuencia con el haplotipo CC-A1 D-Loop previamente reportado para la tortuga caguama del Caribe. Los trece genes que codifican proteínas en el mitogenoma Cc-AO-C se compararon y alinearon con los de otras cuatro tortugas caguama de distintas localidades (Florida, Grecia, Perú y Hawái). Once de estos genes presentaron niveles moderados de diversidad genética, y los genes COII y ND5 mostraron las diversidades nucleotídicas más altas, con un número promedio de diferencias entre pares de secuencias de 6.6 y 25, respectivamente. Adicionalmente, se llevó a cabo la primera aproximación relacionada con el análisis de la estructura 2D y 3D de t-RNAs en este mitogenoma, lo cual condujo a la observación de características únicas en dos tRNAs ($tRNA^{Trp}$ y $tRNA^{Leu}$). La filogenia de las tortugas marinas fue revisada a la luz de la nueva información mitogenómica. El mitogenoma, así como los genes individuales ND5, ND4 y 16S, proporcionan datos filogenéticamente informativos. En conclusión, este estudio resalta la importancia de los datos del mitogenoma para revelar procesos de flujo génico en las poblaciones naturales de tortuga caguama, así como para entender la historia evolutiva de las tortugas marinas.

Palabras clave: mitogenome; *Caretta caretta*; cheloniidae; coding genes; sea turtle phylogeny.

Genoma mitocondrial de tartaruga-cabeçuda do Caribe colômbiano completo: análise de estrutura de tRNA e filogenia revisada de tartarugas marinhas

Resumo. A tartaruga marinha *Caretta caretta* (Cc) é uma espécie amplamente distribuída e ameaçada de extinção que enfrenta um declínio crítico da população, especialmente nas colônias do Caribe colombiano. Marcadores moleculares, como sequências de DNA mitocondrial (mtDNA), são de grande importância para a descrição, monitoramento e análise filogenética de populações migratórias de tartarugas. Este estudo mostra a obtenção e análise do genoma mitocondrial de uma tartaruga-cabeçal Cc aninhada na costa Caribe da Colômbia. O genoma mitocondrial é constituído por 16.362 pb, com uma região não codificante (D-Loop), 13 genes codificadores de proteínas (13 PCG), 22 genes tRNA e 2 rRNA (16S e 12S) e uma frequência nucleotídica de T: 25.7 % , C: 27 %, A: 35 % e G: 12,2 %, todos organizados de forma semelhante à maioria dos mitogenomos de vertebrados. Esta tartaruga Cc colombiana apresentou um novo haplótipo D-Loop com treze sítios polimórficos quando comparado ao haplótipo CC-A1.1 (96 %). Além disso, onze genes codificadores de proteínas entre as tartarugas marinhas de diferentes origens apresentaram uma diversidade genética semelhante, exceto os genes COII e ND5 que apresentaram o maior número médio de diferenças entre pares de seqüências (16.600 e 25.000, respectivamente). Aqui relatase a primeira abordagem relacionada à análise de estruturas 2D e 3D para Cc e descrevese as diferenças em dois tRNAs ($tRNA^{Trp}$, $tRNA^{Leu}$). As inferências bayesianas e os métodos de máxima verossimilhança explicam melhor a filogenia das tartarugas marinhas quando utilizamse mitogenomes completos, assim como os genes ND5, ND4 e 16S. Os genes marcadores ATP8, ND4L e ND1 apresentaram relação filogenética pouco suportada. Como conclusão, este estudo apresenta o uso de mitogenomes completos como uma alternativa para melhorar a análise filogenética em tartarugas marinhas e é a primeira análise genética de mitogenomes completos de nidificação na Colômbia.

Palavras-chave: mitogenoma; *Caretta caretta*; cheloniidae; genes codificadores; tartaruga marinha, filogenia.

Katherin Eliana Alejandra Otálora Acevedo

Her research interest is the analysis of evolutionary patterns in amphibians and reptiles, based primarily on the use of molecular tools and population genetics to delineate evolutionary units, management units in conservation processes, gene flow, determining connectivity patterns through the landscape and historical processes that could leave evolutionary marks on the demographics and the current geographical distribution of organisms.

Javier Hernández Fernández

Associate professor in the Department of Natural and Environmental Sciences at the Jorge Tadeo Lozano University in Bogota for 12 years. During these years he has taught genetics and molecular biology. Leader of the Research Group "Genetics, Molecular Biology and Bioinformatics". He is a PhD candidate at the Pontificia Universidad Javeriana and is currently working on the ecotoxitranscriptomics of the marine turtle *Caretta caretta*.