

PHYLOGEOGRAPHY OF THE LOGGERHEAD TURTLE *Caretta caretta* (TESTUDINES: CHELONIIDAE): FIRST CASE OF STUDY FOR THE COLOMBIAN CARIBBEAN

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Introduction

The loggerhead sea turtle (*Caretta caretta*) is distributed globally along tropical and subtropical latitudes, their complex life cycle that includes extensive migrations and late sexual maturity combined with anthropogenic threats (Eckert *et al.*, 2000) turned this seaturtle status to threatened since 1996 (IUCN, 2010). Molecular markers inherited maternally such as control region (mtDNA) are related; considering the lacking of information for the Colombian Caribbean a haplotypic characterization of the loggerhead turtle was performed in two regions of the Atlantic coast of Colombia; Don Diego beach (Nesting area) and San Martin de Pajarales Island (Feeding ground) using direct sequencing and phylogenetic inference.



Figure 1. Loggerhead turtle *Caretta caretta* (Picture taken by Carolina Franco)

Materials and Methods

During years 2008 and 2009, a survey of peripheral blood samples was performed with eight loggerhead turtles of the Colombian Caribbean. Genomic DNA was extracted from lymphocytes using a UltraClean™ Tissue and Cells commercial kit, mitochondrial control region (D-loop) was PCR-amplified using primers TCR-5 and TCR-6 (Norman *et al.*, 1994), subsequently this products were purified and directly sequenced at Macrogen Inc. (Seoul, Korea). A basic local alignment (BLAST- GenBank) was performed to identify the haplotypes in Colombian aggregations. In order to perform phylogenetic inference, these sequences were assembled and aligned using Bioedit Sequence Alignment Editor 7.0 software (Hall,1999), phylogenetic analysis was performed using Maximum Parsimony (PAUP 3.0) (Swofford, 1991) and Maximum-Likelihood (Rax-ML Cipress 2.0) (Starmatakis *et al.*, 2005) criteria. Statistical support was provided for phylogenetic trees with a bootstrap analysis (Felsenstein, 1985).

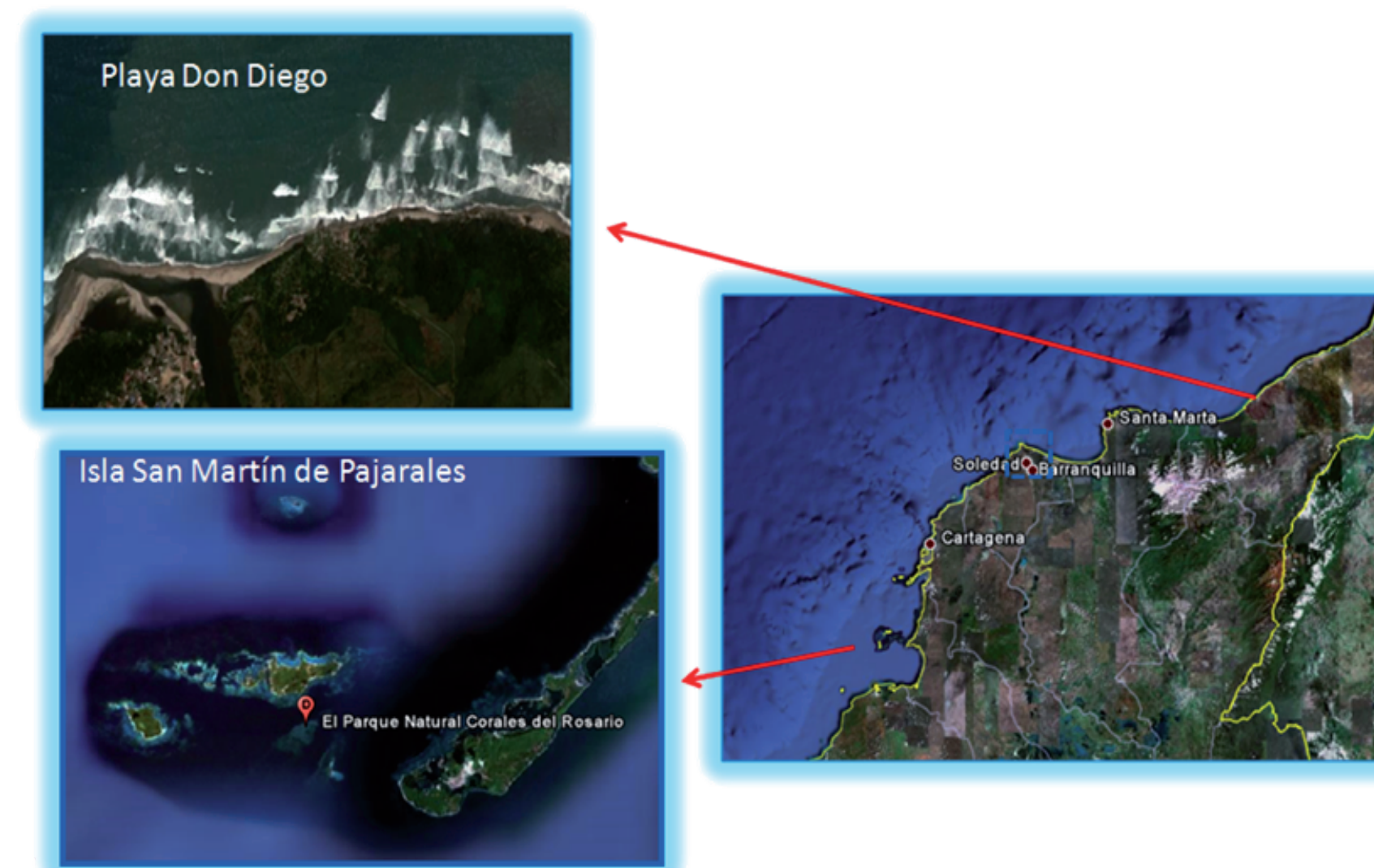


Figure 2. Geographic map of the juveniles survey locations: Upper - San Martín de Pajarales Island, National Natural Park Corales del Rosario and San Bernardo (PNNCRSB). Lower - Don Diego Beach, Magdalena.

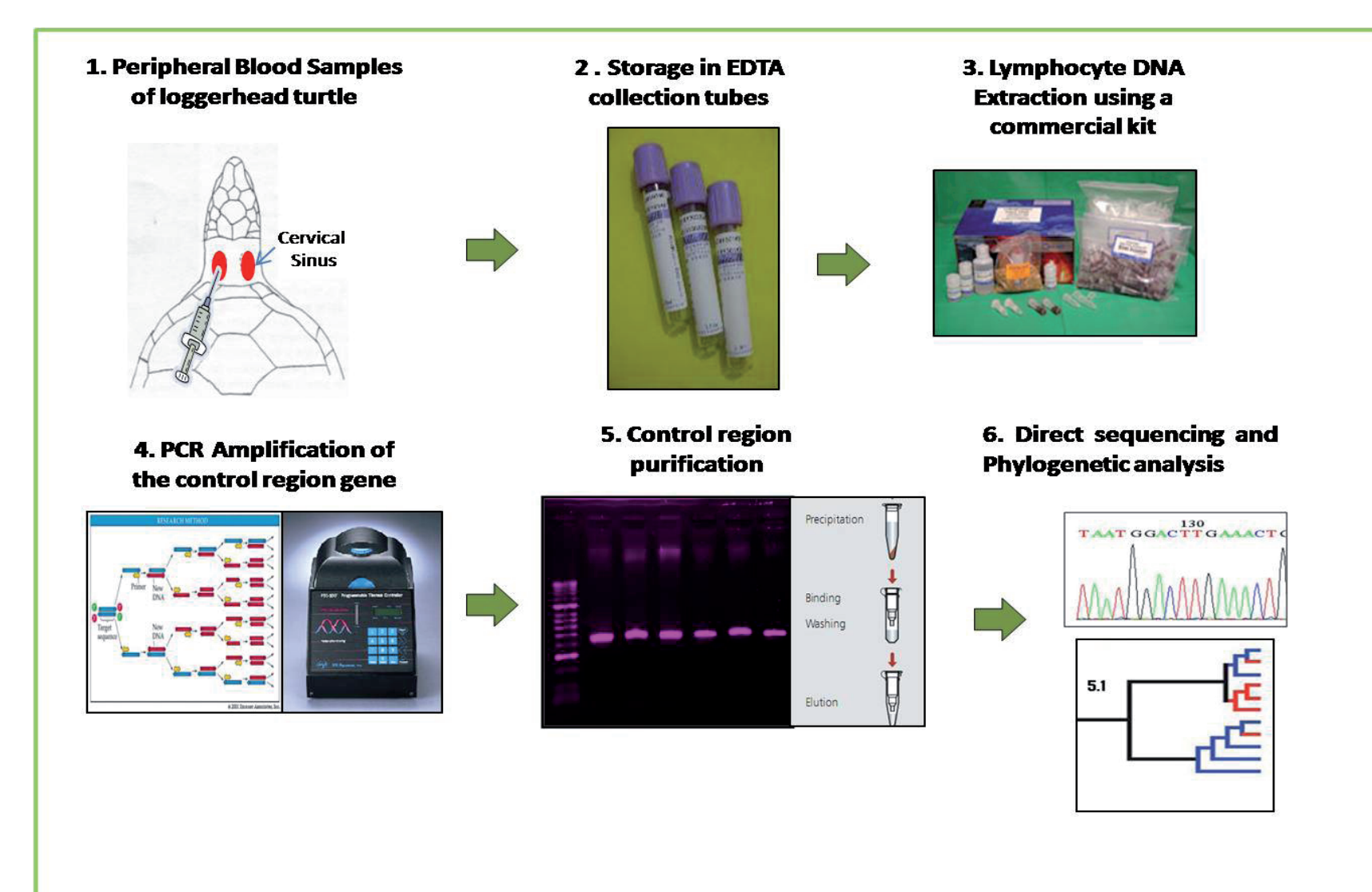


Figure 3. Diagram of the methodology used in this assessment. 1. Peripheral blood samples of loggerhead turtles. 2. Blood storage in collection tubes. 3. DNA extraction using a commercial kit. 4. PCR amplification of the control region gene. 5. Direct sequencing and phylogenetic analysis.

Results

PCR products of control region (mtDNA) with a molecular weight of ~398 bp were obtained from eight loggerhead turtle juveniles of the Colombian Caribbean. Two previously reported haplotypes were identified in these individuals: **CCA1** (50% - N=4) present in nesting colonies of South Florida (U.S), Mexico and Mediterranean sea, and **CCA2** (37,5% - N=3) registered in North Florida, Georgia and South Carolina nesting colonies, also a new sequence **CCDD1** (N=1 - 12,5%) (**Table 1**) found in an individual corresponding to Don Diego nesting beach, Colombian Caribbean, displaying similarity to CCA1 (90%).

Table 1. Haplotypes identified in the present assesment (SFL = South Florida, U.S.A, MX = Mexico, BRA = Brasil, MT = Mediterranean sea, NFL = North Florida U.S.A, CS = South Carolina, U.S.A, GA = Georgia, U.S.A.

Haplotypes	Frequency in this study	Nesting Col.
CC-A1	50%	SFL, MT & BRA
CC-A2	37.5%	NFL, CS & GA
CCDD1	12.5%	Colombia

Phylogenetic analysis (MP and ML) displays a relationship of nesting and feeding aggregations of Colombia with major populations in the Atlantic and Mediterranean. Nesting aggregation present in Colombia is related to nesting colonies in South Florida (U.S), Mexico and Greece. Loggerheads turtles from feeding ground in Colombia are grouped with other aggregations of feeding populations from North Atlantic, Mediterranean sea (Spain and Italy) also sequences reported frequently from nesting populations in North Atlantic and Mexico. This pattern suggests that individuals that use Colombian Caribbean for feeding and reproductive activities are part of an Atlantic metapopulation, where sequences CCA1 and CCA2 are the most frequent and provides juveniles to feeding grounds.

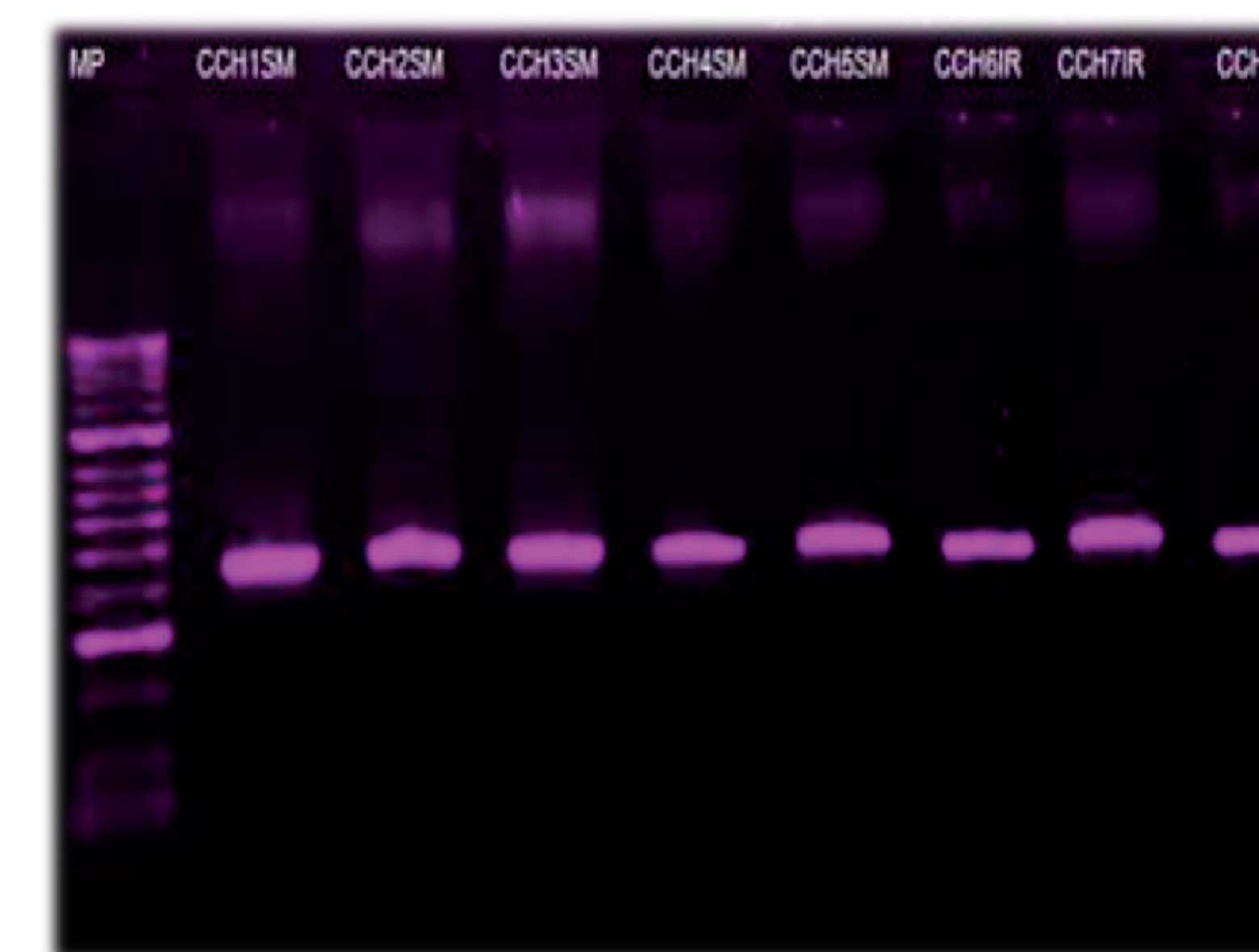
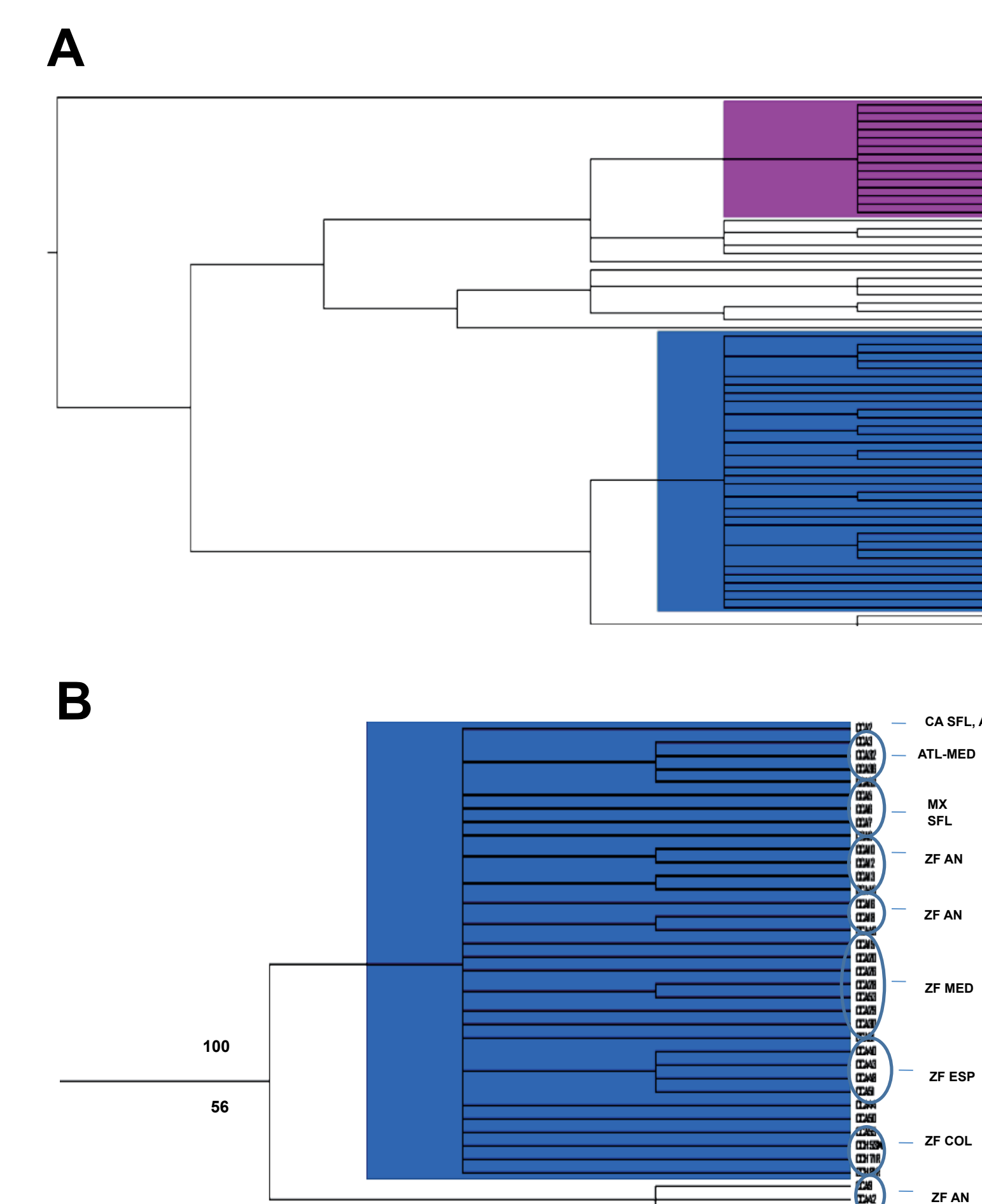


Figure 4. Control region PCR products revealed in a 2% agarose gel.



Conclusions

Supperaggregations in Southeastern U.S.A and Mediterranean could be the nesting aggregation evaluated in Colombia, by means of recent colonization events (Bowen & Karl, 2007), helped by the strong migratory behavior of loggerhead turtles and marine currents such as Caribbean, Gulf and North Atlantic Gyre. Due to the preserence of a new haplotype of *C. caretta* in the Colombian Caribbean we suggest the fulfilling of natal homing hypothesis.

Literature Cited

Ceballos, C. 2003. Distribución de playas de anidación y riesgos para la conservación de las tortugas marinas en el Caribe colombiano. *Boletín de Investigaciones Marinas y Costeras*. Vol. 33. pp 79 - 99.
 Eckert, K. L., Bjørndal, K. A., Abreu-Grobois F. A., Donnelly M. (EDITORES). 2000 (Traducción al español). *Técnicas de Investigación y Manejo para la Conservación de las Tortugas Marinas IUCN/CSE*. Publicación No. 4.
 Encalada, S., Bjørndal, K., Bolten, A., Zurita, J., Schroeder, B., Possardt, E., Sears, C., Bowen, B. 1998. 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: 587 - 575.
 Hall, T. A. 1999. BioEdit a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Symp Ser* 41: 95 - 98.
 IUCN 2010. IUCN Red List of Threatened Species. Version 2010.1. <www.iucnredlist.org>. Downloaded on 25 May 2010.

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For further information

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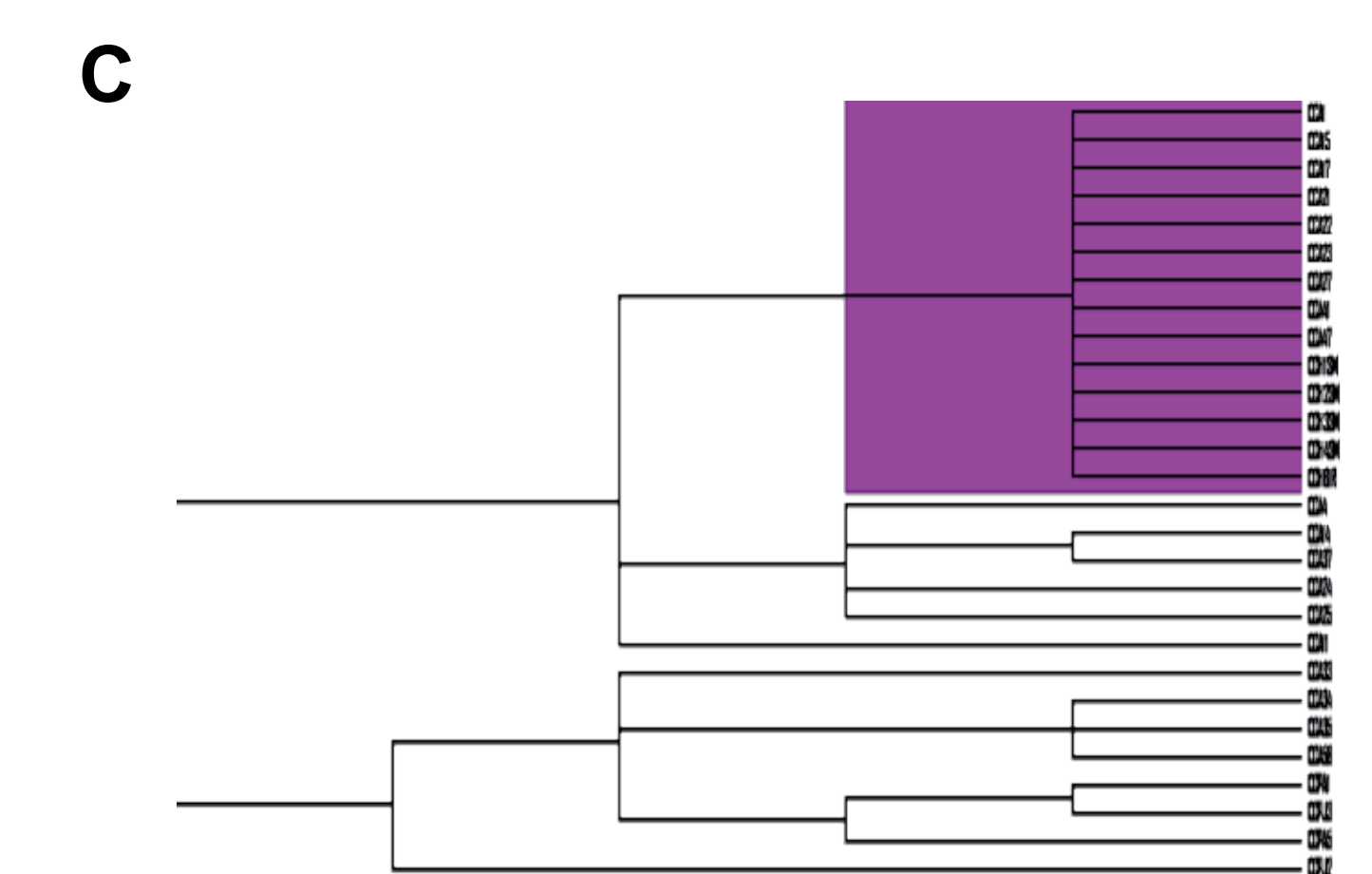


Figure 5. MP and ML Consensus Tree. Displays the relationship of the nesting and feeding aggregation in Colombia with other main populations in the Caribbean, North atlantic and Mediterranean sea. (SFL MX GR= Nesting colonies of U.S.A, Mexico and Greece, CA COL= Colombia, E.U = Nesting colony from U.S.A, CA BR = Brazil, CA PAC = Pacific colonies, ZF AN = North Atlantic, ZF BR= Brazil, ZF MED = Italy, ZF ESP= Spain, ATL-MED= Shared between Mediterranean and Atlantic.