RAPD-PCR TO DETERMINE THE GENETIC DIVERSITY OF THE LOGGERHEAD TURTLE *Caretta caretta* (TESTUDINES: CHELONIIDAE)

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Introduction:

The loggerhead sea turtle, *Caretta caretta* is a widely distributed species found from Rio de la Plata (Argentina) to Murmansk (Russia) (Garcia *et al.,* 2002). Among others, nesting populations of *Caretta caretta* along the beaches of the Colombian Caribbean. Due to human pressures, such as habitat degradation (Eckert, 2000), bycatch from industrial and subsistence fisheries (Camiñas, 2006) and the marketing of its eggs, meat, and oil, this species is listed as endangered (IUCN, 1996). This situation has prompted the development of national laws (INVEMAR, 2002) and several international treaties (including CITES) to conduct scientific studies in order to support management and conservation of this species. In this study, we applied an optimized RAPD-PCR technique, and identified preliminarily biodiversity indices that enabled us to recognize variations at the genetic level of two groups of sea turtles from two Colombian beaches, Don Diego and Islas del Rosario. We provide a framework for the study and conservation of the species in these particular areas. This is the first molecular-genetic study conducted in the Colombian Caribbean concerned with the management and conservation of *Caretta caretta*

Materials and Methods:

Figure 1: Map of the Study site. Islas del Rosario near Cartagena and Don Diego Beach in Santa Marta to obteinedsamples inmadure loggerhead Turtle *Caretta caretta*

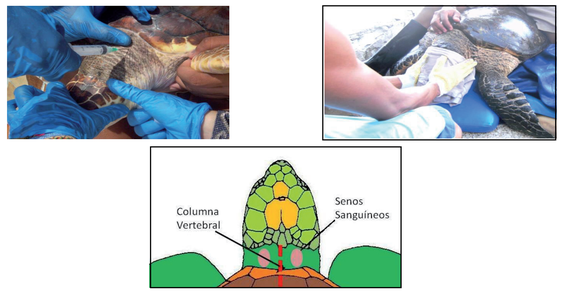


Figure 2: Blood was colected to cervical or caudal Vein, according methodology Dutton (1996)

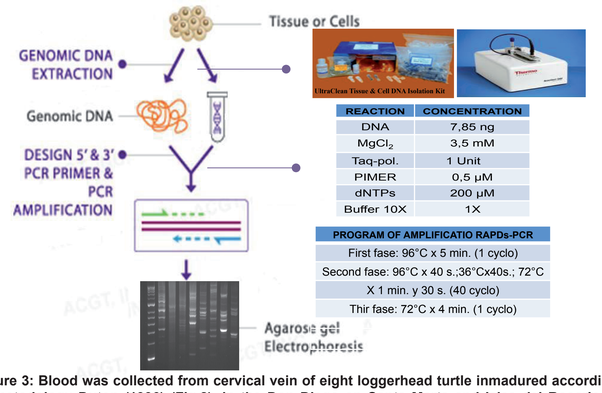


Figure 3: Blood was collected from cervical vein of eight loggerhead turtle inmadured according to metodology Duton (1996) (Fig.2), in the Don Diego on Santa Marta and Islas del Rosario in Cartagena(Fig.1.). The nuclear DNA was isolated from the blood samples by UltraClean Tissue & Cells DNA Isolation Kit according to speceification of MO BIO laboratories, Inc. USA. To detect genome variability, nine Primers were used: OPA1, OPA2, OPA3, OPA4, OPA5, OPA6, OPA8, OPA9 and OPA10 (Operon Technologies Inc. Almeda CA, USA) the best Primer used to construct binary matrix (present (1) and absence (0)) to determine genetic diversity with PRIMER 5 program and Shannon diversity index (H) was also calculed, Margalef richness (d), Simpson (1-Lamda) and Pelou Uniformity (J). Dendrogram was constructed by Bray-Curtis similarity for search intra and interspecif relation between two population.

Results:

Using blood samples from eight loggerhead turtle. *C. caretta* collected from beaches in Don Diego (N=5) and Islas del Rosario (N=3), we isolated and quantified the DNA at a concentration of 40-111 ng/ml (Fig. 4). We used the Taguchi method (Codd and Clarkson, 1994), which reducs the number of RAPD-PCR reactions (Fig. 5 and Tab.1.). Optimal conditions for the RAPD-PCR reaction for *C. caretta* were obtaind using a concentrtion of 3.5 mM $MgCl_2$ (Fig. 6B.), 0.5 µM of Primer (Fig. 7A.) and one unit of Taq DNA polymerase (Fig. 7B.)

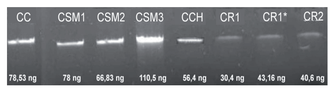


Figure 4: DNA quality and concentration of each samples of loggerhead turtle

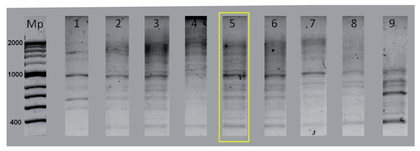
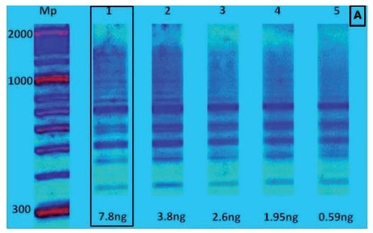


Figure 5: RAPD profile from amplification through theTaguchi method, of fragment of genomic DNA loggerhead turtle

Table 1: Different concentration to maximizing the Taguchi method, using DNA loggerhead turtle and throug amplification for RAPD

REACTION	PRIMER (µM)	DNA (ng)	$MgCl_2$ (mM)	Taq-pol (Uni)
1	0,25	3	3	2
2	0,375	6	2,5	2
3	0,5	9	2	2
4	0,25	6	2	3
5	**0,375**	**9**	**3**	**3**
6	0,5	3	2,5	3
7	0,25	9	2,5	5
8	0,375	3	2	5
9	0,5	6	3	5



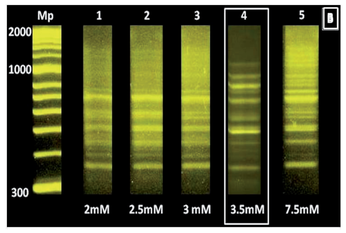
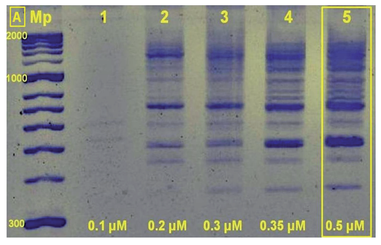


Figure 6: A) RAPD profile from amplification with diferent concentration of DNA loggerhead turtle. B) Agarosa gel show a different concentration of Magnesium Cloride ($MgCl_2$)



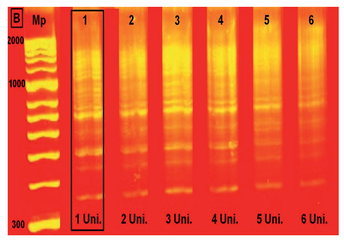


Figure 7: A) Variation of concentration Primer with Primer OPA5 in RAPD amplification B) Agarosa gel profile show a different concentration of Taq polimerasa

Genetic diversity, determined as band richness (Margalef index), ranged between 3.64 to 4.43 for turtles form Don Diego and 2.16-1.44 for turtles from Islas del Rosario. Genetic diversity, measured as Shannon diversity was low (2.48-0.69) (Tab.2), probably as a result of small population size as a result of different human pressures operating on this species. The dendrogram obtained by RAPD-PCR revealed a high similiarity between turtles nesting on Don Diego beach (85%) and Islas del Rosario (100%). One turtle nesting on Don Diego beach showed greater similarity to *C. caretta* of Islas del Rosario (44%) than with other turtle from Don Diego. One explanation for the higher similarity observed between groups than within groups might be that all turtles from part of the same population that feeds on Island del Rosario and nests on Don Diego beach (Fig.9)

Results:

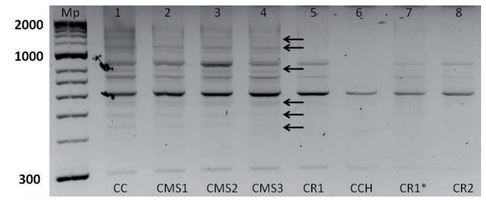


Figure 8: Agarosa gel show RAPD profile from amplification to best Primer OPA4 to determine variation in two population of loggerhead turtle

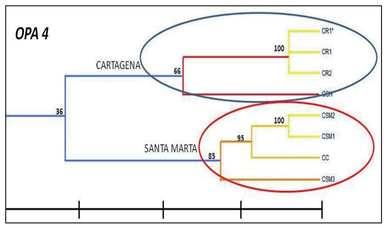


Figure 9: Cluster tree based on similarity Bray-Curties index of two population of loggerhead turtle using UPGM

Table 2: Different index of biodiversity on two population of loggerhead turtle

SAMPLES	MARGALEF	PIELOU	SHANNON	SIMPSOM
CC	4,4268	1	2.4849	1
CSM1	4,1703	1	2,3979	1
CSM2	4,1703	1	2,3979	1
CSM3	3,641	1	2,1972	1
CR1	2,164	1	1,3863	1
CCH	1,4427	1	0,69315	1
CR1*	2,164	1	1,3863	1
CR2	2,164	1	1,3863	1

Conclusion:

The analysis of RAPDs-PCR using the Taguchi method is suitable method for analyzing genetic diversity in loggerhead turtle (*Caretta caretta*) populations. Optimum conditions for *C. caretta* for the RAPDs-PCR reaction were: 7.85 ng DNA, 3.5 mM $MgCl_2$, 1 unit of Taq DNA polymerase, 0.5 µM of Primer, 200 mM dNTPs in a final reaction volume of 20 µl. The amplification program used included, 96°C for 40 s, 36°C for 40 s and an elongated phase of 72°C for 1.5 min. There were 263 bands with nine primers. The OPA 4 primer produced the largest number polymorphisms (30) among loggerhead turtles. Biodiversity indices showed poor differentiation within the two groups and low genetic diversity for turtles from Don Diego and Islas del Rosario. The low genetic diversity observed is likely to be aproduct of a reproductive bottleneck in *C. caretta* caused by anthropogenic pressures that affect turtles in this area of the Colombian Caribbean. within groups might be that all turtles from part of the same population that feeds on Island del Rosario and nests on Don Diego

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