Preliminary Results Report

STRUCTURE AND GENE FLOW OF THE MORELET'S CROCODILE (*CROCODYLUS MORELETII*) IN MÉXICO

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The project focuses on conservation problems in a priority species such the Morelet's crocodile (*Crocodylus moreletii*) along its natural distribution. The main objetive of the project is to determine the genetic status of Morelet's crocodiles to assess possible problems they are facing and that could endanger the survival of this species. Their populations are considered as "conservation dependent" according to the IUCN, and studies on this species are considered high priority according to the Action Plan proposed by the Crocodile Specialist Group (CSG) of IUCN.

OBJECTIVES

General objective

- Determine the structure and gene flow using microsatellite markers and mitochondrial genes in populations of *C. moreletii*.

Particular objectives

- Determine recent bottlenecks in populations of *C. moreletii* along their natural range as a result of over-explotation in the past.
- Determine de structure of *C. moreletii* in the populations surveyed.

- Determine if there is gene flow between populations of *C. moreletii*.

RESULTS AND EXPECTATIONS

Fieldwork. During the period 2009 – 2012 there have been eight surveys to different places where the species occurs in México to collect samples. Addition to taking samples for genetic analysis were obtained important data of the density of this species in two regions (Veracruz and Tamaulipas), these population census data were analyzed and I wrote an article called “Abundance and habitat preference of *Crocodylus moreletii* in the coast of Gulf of Mexico” and it was submit to *Herpetological Conservation & Biology Journal*, I’m awaiting comments from the editor.

Laboratory work. I have 123 samples of blood and tissue of *C. moreletti*, the individuals were selected for genetic analysis of populations geographically remote from each other, the neonates and individuals with a total length < 80 cm were excluded. I have 250 mitochondrial sequences of 75 samples and I’m sequencing another 10 individuals more (Table 1). For the microsatellite analysis I have a total of 90 individuals of three important populations (Table 2), and I analyzed 279 allelic frequencies with six microsatellites, I´m working with the other samples.

The primers L15459 (Glenn et al. 2002) and CR2HA (Ray and Densmore 2002) designed for crocodiles were used, and I designed three new primers *Cyt B*, *Cyt C* and *NADH2* to amplified and sequenced ~630 pb, ~923 pb and ~680 pb respectively.

Table 1. Number of samples sequenced with each mitochondrial marker

		C. Reg.	**NADH2**	**Cyt B**	**Cyt C**
Location	*n* sampled	*n* sequenced	*n* sequenced	*n* sequenced	*n* sequenced
Veracruz	24	22	22	22	10
Tamaulipas	21	20	20	20	3
Q. Roo	15	13	13	13	6
Chiapas	15	13	13	13	3
Tabasco	10	7	7	7	3
TOTAL	**85**	**75**	**75**	**75**	**25**

Table 1. Number of samples sequenced with each microsatellite marker

		cj16	**cj18**	**cj35**	**cj109**	**cj119**	**cj131**
Location	*n* sampled	*n* sequenced	*n* sequenced	*n* sequenced	*n* sequenced	*n* sequenced	*n* sequenced
Veracruz	30	20	20	20	20	11	11
Tamaulipas	30	15	15	15	15	15	15
Quintana Roo	30	15	15	15	15	15	12
TOTAL	**90**	**50**	**50**	**50**	**50**	**41**	**38**

The estimates of haplotype and nucleotide diversity are indicated in Table 3, although some samples left for to analyze. With this preliminary data I can observe that the diversity is according with other studies of genetic in this species as Cedeño-Vázquez (2008) and Ray et al. (2004). In the Table 4, the values of F_{TS}, indicate a genetic subdivision between populations.

Table 3. Preliminary data of genetic diversity inc*C. moreletii*

Gene	Locality	Haplotype diversity	Nucleotidy diversity	Tajima *D*	Fu *Fs*
Control Reg.	Veracruz	0.600 ± 0.215	0.006 ± 0.002	-0.977	2.242
	Tamaulipas	0.714 ± 0.127	0.018 ± 0.010	-1.423	6.501
	Q. Roo	0.857 ± 0.108	0.010 ± 0.002	-0.276	1.393
	Chiapas	0.286 ± 0.196	0.006 ± 0.004	-1.622	4.561
NADH2	Veracruz	0.634 ± 0.142	0.015 ± 0.007	-1. 532	5.732
	Tamaulipas	0.228 ± 0.154	0.004 ± 0.005	-0. 356	1.987
	Q. Roo	0.787 ± 0.174	0.022 ± 0.010	-0.786	2.653
Cyt B	Veracruz	0.750 ± 0.139	0.137 ± 0.007	-1.444	2.742
	Tamaulipas	0.750 ± 0.139	0.003 ± 0.001	-1.044	0.261
	Q. Roo	0.933 ± 0.122	0.004 ± 0.001	-1.390	-1.786
	Chiapas	0.714 ± 0.181	0.003 ± 0.001	-1.524	-0.428
Cyt C	Veracruz	0.543 ± 0.109	0.018 ± 0.006	-1.212	-0.917

Table 4. Preliminary data of F_{ST} to estimate gene flow between populations of *C. moreletii*

Locality	*C. Reg*	*NADH2*	*CytB*	*CytC*
Chiapas - Veracruz	0.250	0.025	0.108	-
Chiapas - Tamaulipas	0.306	0.142	0.027	-
Chiapas - Q. Roo	0.014	0.333	0.043	-
Veracruz - Tamaulipas	0.030	0.000	0.098	-
Veracruz - Q. Roo	0.269	0.033	0.139	0.230
Tamaulipas - Q. Roo	0.382	0.200	0.166	-

On the other hand, with the data of the microsatellite markers, I found extensive variation H_O/H_E because some samples are not integrated yet in the

analysis. In the Table 3 are the data so far about this, but, still needs to include the other DNA samples in the analysis.

Table 3. Preliminary data of microsatellites generally in *C. moreletii*

Locus	GD	H_O	H_E	PCI	Ae
Cj16	0.728	0.889	0.771	0.684	3.682
Cj18	0.602	1.000	0.648	0.523	2.513
Cj35	0.278	0.333	0.333	0.239	1.385
Cj109	0.643	1.000	0.692	0.585	2.800
Cj119	0.776	1.000	0.835	0.744	4.455
Cj131	0.228	0.000	0.303	0.239	1.385
Mean	0.551	0.704	0.597	0.502	2.703

GD= Genetic Diversity, H_O=Heterozygosity observed, H_E= Heterozygosity expected, PCI= Polymorphic Content Index, Ae= Effective alleles

Preliminary data show clear trends on the genetic structure of populations of the Morelet's crocodile, exist an evident genetic subdivision between the north and south populations in Mexico, and in some regions there are a low genetic variability.

In December of this year, I finish the laboratory work and I will start write the article in January 2013.

CHECKOUT OF CHARGES RESULTING FROM LABORATORY WORK

Item	Cost *US*	Total *US*
Microsatellite primer	$ 80	$ 1,440
Mitochondrial primer	$ 30	$ 240
Microsatellite sequence	$ 7	$ 1,953
Mitochondrial sequence	$ 7	$ 1,750
TOTAL		**$ 5,383**