

Title:

GENETIC DIVERSITY, POPULATION SUBDIVISION AND GENE FLOW IN  
MORELET'S CROCODILE (*CROCODYLUS MORELETII*) FROM BELIZE, CENTRAL  
AMERICA

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The lack of information surrounding natural history and ecology of the endangered Morelet's crocodile (*Crocodylus moreletii*) has prompted a baseline study of the population genetics for this species. Nine microsatellite loci have been used to estimate genetic structure within and gene flow patterns among crocodiles (using a recently described maximum likelihood approach) from seven localities in north-central Belize. Individuals from the seven localities grouped into four apparent populations. Within localities, a high degree of genetic heterogeneity was observed. Among all localities, some subdivision was present ( $F_{ST} = 0.062$ ;  $R_{ST} = 0.100$ ). Furthermore, among the apparent populations, we found a significant correlation between geographic distance and genetic subdivision. Our findings suggest a relatively high level of migration among populations ( $Nm = 5.15$ ) and are consistent with an isolation-by-distance model of gene flow. Two contiguous sub-populations in particular, New River and New River Lagoon, may form an important source for genetic variation for smaller populations throughout the region. These data will allow us to test hypotheses of relatedness among *C. moreletii* for other drainages in Belize and will be useful in optimizing future management programs for *C. moreletii*.

Due to increases in habitat destruction and fragmentation worldwide, conservation biologists have begun to recognize the significance of maintaining biodiversity (Erwin, 1991). Preservation of necessary habitat in natural areas is one important step in species conservation and management. Not as widely recognized, but potentially just as critical for ensuring long-term survival, is the preservation of species' genetic structure at the population level (Frankel and Soulé, 1981). Over the past decade, researchers have placed increasing emphasis on the role that genetics plays in wildlife preservation and using genetic information to preserve variation within species (Haig, 1998). Simply stated, increased genetic variation within local populations may enhance species' ability to adapt to changing environmental conditions (Mayr, 1963).

Present day crocodylians (crocodiles, alligators, caimans and gharials) come from an ancient and diverse lineage, once comprised of over 125 genera (Romer, 1956). As keystone species, crocodylians play an important role in the biodiversity and maintenance of their ecosystems (Kohn, 1989; Ross, 1998). Yet, our knowledge of crocodylian population status and ecology is "poor" or "extremely poor" (Ross, 1998) for more than half of the extant species. One such species, Morelet's crocodile (*Crocodylus moreletii*), inhabits the lowlands of the Yucatan Peninsula in Mexico, Belize and Guatemala (Lee, 1995). In Belize, *C. moreletii* is primarily found in freshwater marshes, ponds, lagoons and rivers (Abercrombie et al., 1980; Platt, 1996; Platt and Thorbjarnarson, 2000). Populations of *C. moreletii* declined to critically low levels in the late 1960's and the species was ultimately classified as endangered (Ross, 1998). Although hunting pressure is not currently as intense as in the recent past (Platt and Thorbjarnarson, 2000), animals continue to be killed occasionally by locals who consider them a physical threat.

Overall, populations of *C. moreletii* appear to be recovering (Platt, 1996; Platt and Thorbjarnarson, 2000). However, there is literally no information on the population genetics of this species.

Previous studies have revealed relatively low levels of genetic diversity among alligator and crocodile populations. Gartside et al., (1977), Menzies et al., (1979) and Adams et al., (1980) each found very low levels of genetic variation (heterozygosity and proportion of polymorphic loci) within populations of American alligators (*Alligator mississippiensis*). Lawson et al., (1989) reported similar findings for Nile crocodile (*C. niloticus*) populations. However, isozymes were used as molecular markers in these studies and diversity estimates were based on a low number of polymorphic loci. Since most of the variation detected in non-denaturing protein electrophoresis reflects changes in charge of amino acids on the surface of the molecule, estimates of mutation rates for isozymes are almost always low (Queller et al., 1993; Mitton, 1994). This makes it more difficult to estimate the genetic variation that actually exists in populations.

Glenn et al., (1998) recently illustrated the potential of microsatellites for genetic analyses of crocodylian populations. They reported significant differentiation between populations of *A. mississippiensis* between Florida and Louisiana. Furthermore, they found that within-population heterozygosity levels (averaging 0.47) were much higher than isozyme and allozyme studies had previously reported. This was recently corroborated by another study of populations of *A. mississippiensis* (Davis et al., 2001) in which microsatellites were employed. Davis et al., (2001) reported a high level of genetic variation for six populations located throughout the southeastern United States. The nature of microsatellite evolution appears to facilitate population level

analyses (Jarne and Lagoda, 1996; Moxon and Willis, 1999), and these findings support the use of microsatellite analyses as a means of measuring genetic structure both within and among crocodilian populations.

The primary objectives of this research were to identify the genetic structure of several Morelet's crocodile localities in north-central Belize and to estimate levels of genetic diversity within and among these localities, ultimately providing a way to assess the amount of gene flow among genetic units. New River and New River Lagoon together make up a significant watershed within a relatively undisturbed area that includes the largest freshwater system in Belize (Fig. 1). This watershed houses a rich variety of flora and fauna, and supports relatively high densities of *C. moreletii* (Rainwater et al., 1998; Platt and Thorbjarnarson, 2000). Additionally, several other localities (representing a range of slightly different habitats) were also sampled.

## MATERIALS AND METHODS

Study area.-Fieldwork was conducted in north-central Belize, and crocodiles sampled from seven localities were included in this study (Fig. 1): 1) Indian Creek Lagoon (IC; 17°45'N, 88°43'W), 2) Habenero Lagoon (HAB; 17°36'N, 88°43'W), 3) Cox Lagoon (COX; 17°50'N, 88°37'W), 4) Banana Bank Lagoon (BB; 17°20'N, 88°47'W), 5) New River (NR; 17°50'N, 88°37'W), 6) New River Lagoon (NRL; 17°42'N, 88°38'W), and 7) Gold Button Lagoon (GBL; 17°55'N, 88°45'W). These localities range from large river systems (NR/NRL) to alluvial (COX) and non-alluvial (IC, HAB, BB, GBL) lagoons of various sizes and crocodile densities (Rainwater et al., 1998; Platt and Thorbjarnarson, 2000).

Sample collection.- Samples were collected over a two-year period (1998-99), primarily from May to September. Crocodiles were spotted at night, and captured using the break-away snare technique (Hutton, 1989). Approximately 1 ml of blood was collected from the dorsal sinus of each animal, just posterior to the head (Olson et al., 1975; Bayliss, 1987) and then placed in an extraction buffer (ACD-B, see White and Densmore, 1992). Individuals were sexed by cloacal examination of the genitalia (Allsteadt and Lang, 1995; Platt, 1996). Measurements of total length, snout-vent length and body mass were recorded. Following sample collection, each crocodile was marked by a numerical scute clipping system and released at the site of capture. Location coordinates were recorded using a Magellan 2000 hand-held global positioning system (GPS). These GPS coordinates were used to directly measure movement for recaptured individuals.

DNA isolation and microsatellite amplification.-DNA was isolated from blood samples using the PURGENE™ DNA isolation kit (Gentra). Nine dinucleotide microsatellite loci were amplified using polymerase chain reaction (PCR) reverse and forward primers (*Cj* 16, *Cj* 18, *Cj* 20, *Cj* 35, *Cj* 109, *Cj* 119, *Cj* 119, *Cj* 127, *Cj* 128, *Cj* 131) characterized in FitzSimmons et al. (2001). To enable the detection and sizing of PCR fragments, one primer from each pair was end labeled with a fluorescent dye group (6-FAM, TET or HEX (Applied Biosystems)). DNA samples were amplified in 25µl reaction volumes containing 2 units of *Taq* polymerase (Fisher), 2.4µl of 10X Fisher reaction buffer, 2.0µl 15µM MgCl<sub>2</sub>, 1.0µl of 10mM dNTP's (Promega), 1.0µl of 10µM of each primer, approximately 100 ng of DNA and water for the final volume. Details for specific thermocycler parameters and annealing temperatures for each primer pair are presented in Dever and Densmore, 2001). To determine base pair size and relative concentration for dilutions, PCR products were visualized in 2.5% agarose (BRL UltraPure™) gels. Samples were then run on an Applied Biosystems Prism 310, using the Tamra standard (PE Applied Biosystems). Results were compiled and analyzed with GENESCAN and GENOTYPER software (Applied Biosystems).

Data analysis.-Genetic structure was analyzed in several ways. Within- and among-locality diversity values were estimated using allele frequencies, observed heterozygosities and unbiased expected heterozygosities (Nei, 1978) using the Matlab (Mathworks 1997) function *hetzyg* of Strauss (unpubl.). Departure from expected values of Hardy-Weinberg equilibrium was tested via Fisher's exact probability test using a version of the Markov-chain random walk algorithm from Guo and Thompson, (1992) in GENEPOP ver. 3.1c, (updated from Raymond and Rousset, 1995), as well as a conventional chi-square goodness-of-fit test. Fisher's exact tests for linkage

disequilibrium among all possible pairs of loci were performed using the web version of LINKDOS (Garnier-Gere and Dillman, 1992).

The extent of subdivision among localities was estimated in two ways. First, analysis of molecular variance (AMOVA) based upon the Cockerham (1973) and Weir and Cockerham (1984) methods (allowing for mutations between haplotypes) was used to estimate genetic-variance components, using Arlequin version 1.1 (Schneider et al., 1997). Second, Weir and Cockerham's (1984) inbreeding estimates [ $F_{IS}$  and  $F_{ST}(\theta_p)$ ]; bootstrapped confidence intervals for the F-statistics (based on 5000 bootstrap iterations) were estimated with the Matlab function *fstat* (Strauss unpubl.). Both of these sets of calculations assume the infinite-allele model of mutation (Wright, 1948; Kimura and Crow, 1964).

Two other measures of population structure based on the stepwise-mutation model were also estimated (Slatkin, 1995). The first measure,  $R_{ST}$  (Michalakis and Excoffier, 1996), which uses the squared size differences between alleles, was estimated using *micsatfs* (Strauss unpubl.). Factoring the variable of microsatellite allele size into calculations for subdivision uses more information, i.e., allele size; therefore,  $R_{ST}$  is the preferred method for resolving genetic structure from microsatellite variation (Slatkin, 1995; Gaggiotti, 1999). The second measure, genetic distance ( $\delta\mu^2$ ) (Goldstein et al., 1995), which is based on the squared differences in mean allele size averaged over loci, was estimated using RST-CALC (Goodman, 1997). Bootstrapped confidence intervals for these statistics (based on 5000 bootstrap iterations) were estimated with Matlab function *micsatfs* (Strauss unpubl.).

Mantel's test (Mantel, 1967) was used (Matlab function *mantel*, Strauss unpubl.) to determine the relationship between geographic distance and each population structure estimate

( $F_{ST}$ ,  $R_{ST}$  and  $\delta\mu^2$ ). The numbers of migrants among localities were estimated from the Barton and Slatkin (1985) private-allele calculation in GENEPOP.

The model-based clustering program STRUCTURE of Pritchard et al. (2000) was used to estimate the underlying population structure of Morelet's crocodile in the region. This procedure uses maximum-likelihood clustering of individuals to infer the number and identities of real biological populations underlying the locality data. The program was first run assuming the crocodiles represented a single underlying population, and the estimated log-likelihood of the data given the model  $\ln[\Pr(X|K)]$  was recorded. This procedure was repeated for models assuming 2–7 populations, respectively, and the model providing the greatest log-likelihood was used to represent the underlying population structure (Table 5). Once that number of populations was identified ( $n=4$ ), an additive neighbor-joining tree (Saitou and Nei, 1987) expressing the relationships among localities in terms of this structure was constructed (Matlab function *addtree*, Strauss unpubl.) from a matrix of Euclidean distances among localities in a four-dimensional space, where the axes of the space represent the proportions of membership of each locality assigned to each of the proposed four populations. In the absence of an outgroup, Farris' (1972) minimum rate-heterogeneity criterion was used to estimate the position of the root.

## RESULTS

Microsatellite variation. -Nine microsatellite loci showed considerable allelic variation within localities (Appendix 1). An average of 8 alleles per locus was found across all localities, with a range of 2.1 to 5.6 alleles per locus (corrected for sample size) observed within localities (Appendix I). Unique (private) alleles for each locus were found at all but one locality. Frequencies for these were relatively low (e.g., *Cj18*-allele a, from NRL; Appendix I).

Due to uncertainty about the distribution of these microsatellite loci within the Morelet's crocodile genome, linkage disequilibrium tests were performed. Of 227 pairwise comparisons for each locus, 10 pairs had individually significant disequilibrium values ( $p < 0.05$ ); we expected 11 pairs to be significant due to chance alone, and when adjusted for the number of comparisons by a sequential Bonferroni test (Rice, 1989), no pairwise comparisons were significant. For samples with 40 or more individuals, only 8 pairs from 199 comparisons were significant ( $p < 0.05$ ), which is fewer than the 10 expected due to chance; these 8 also are not significant in a sequential Bonferroni test. Therefore none of these pairwise comparisons were deemed to be biologically significant. No pairs of loci had significant disequilibrium values across all localities.

Genetic diversity.-Observed heterozygosity ( $H_O$ ) and unbiased expected heterozygosity ( $H_E$ ) were estimated at each locus for each locality (Table 1). No locality appeared to be deficient in heterozygotes. Exact tests for Hardy-Weinberg equilibrium suggested that several locality groups were not in equilibrium due to an excess of heterozygotes, however, these tests are known to be conservative. Corresponding chi-square tests for each locus indicated that one locus in particular, Cj18 ( $p < 0.001$ ), exhibited an excess of observed heterozygotes and could be responsible for the apparent deviation from equilibrium. When (Cj18) was excluded in subsequent chi-square analyses, the localities were no longer significantly different from the expected Hardy-Weinberg values.

Population subdivision and gene flow.-The results of the molecular variance analysis (AMOVA) revealed a high degree of within-locality variation (i.e., over 94% of the variation detected, data not shown). However, there is no evidence of inbreeding within localities ( $F_{IS} = -0.019$ ) (Table 2). Among localities, the estimated number of migrants ( $N_m$ ) was 5.15 per generation after correcting for sample size. Different estimates for overall population structure were obtained from Weir and Cockerham's  $F_{ST}$  ( $\theta_p$ ) and  $R_{ST}$ . The  $F_{ST}$  estimate ( $F_{ST} = 0.062$ ) suggests little subdivision among localities, whereas  $R_{ST}$  suggests a slightly higher level of subdivision ( $R_{ST} = 0.100$ ). However, the mark-recapture data (Table 3) do not support this level of gene flow between two localities in particular (New River and New River Lagoon).

By comparing pairwise  $F_{ST}$  and  $R_{ST}$  values (Table 4), we observed an ostensible relationship between the genetic distances and amount of geographic distance of localities from the NRL. Additional indirect indications of gene flow were obtained by examining correlations

between geographic distance and measures of population subdivision; we compared pairwise estimates of  $\delta\mu^2$ ,  $F_{ST}$  and  $R_{ST}$  to pairwise geographic distances ( $D_G$ ) among populations. An isolation-by-distance pattern of gene flow was supported by randomized Mantel's tests, which yielded significant matrix correlations ( $\delta\mu^2 - D_G = 0.623$ ,  $pr = 0.021$ ;  $F_{ST} - D_G = 0.595$ ,  $pr = 0.032$ ;  $R_{ST} - D_G = 0.674$ ,  $pr = 0.018$ ).

This basic pattern of isolation-by-distance from NRL, with significant amounts of gene flow within and among localities, was further supported by the model-based clustering method of Pritchard et al. (2000). The analysis strongly supported the existence of four spatially overlapping populations (Table 5, Fig. 2), with some individuals at each collection locality allocated to each of the four underlying populations (Table 6). Despite the spatial overlap, in all cases individuals could be assigned to one of these four hypothetical populations with a probability greater than 0.5. A neighbor-joining tree (Fig. 3) of localities based on these probabilities of population membership is consistent with the hypothesis that the NR and NRL localities could represent the source population from which crocodiles at other localities are derived.

## DISCUSSION

Genetic diversity.-The ability of microsatellites to identify individuals and the potential to determine genetic structure within *C. moreletii* populations has clearly been demonstrated. Our microsatellite analyses revealed relatively high levels of heterozygosity ( $H = 0.49$ ). This estimate suggests that even though *C. moreletii* populations in Belize reached critically low levels during the 1940's through 60's (Ross, 1998; Platt and Thorbjarnarson, 2000), there seems to be no evidence of a major genetic bottleneck. Furthermore, the genetic diversity observed in *C. moreletii* was comparable to estimates for alligator populations reported by Glenn et al. (1998; mean heterozygosity = 0.46) and Davis et al. (2001; mean heterozygosity = 0.64). These values are all considerably higher than those determined from isozyme analyses in both alligator ( $H$  ranging from 0.009-0.02; Gartside et al., 1977; Menzies et al., 1979; Adams et al., 1980) and Nile crocodile populations ( $H = 0.011$ ; Lawson et al., 1989). Together the three DNA studies (Glenn et al., 1998, Davis et al., 2001, and the present study) indicate that microsatellites will be useful genetic markers for addressing crocodylian population genetics.

Population subdivision and gene flow.-A fine-scale analysis of *C. moreletii* sampled from each of these collection localities revealed an overall lack of genetic subdivision within localities. Typically, one indication of inbreeding and subdivision is a decrease in the number of observed heterozygotes. This situation was never observed in the present study; on the contrary, based on the Pritchard et al., (2000) analyses (Table ; Fig. 2), one would predict a large number of heterozygotes at each locality. This apparent lack of subdivision could be related to movement of individuals within deme-like assemblages (subpopulations). In addition, AMOVA and  $F_{(IS)}$ -

statistics estimated for these subpopulations suggested that there was not extensive inbreeding at any locality. Together, these findings suggest frequent movement of individuals within localities. Comparable values were observed in a preliminary study of Nile crocodile populations, where  $F_{IS}$  values for two allozyme loci (-0.12, -0.05) also indicated random mating within populations (Lawson et al., 1989).

Mark-recapture data do not reflect the apparent level of genetic exchange that is going on among localities. A possible explanation for such a discrepancy could be that most recaptured individuals were not of breeding size, and perhaps younger crocodiles (< 5 years old) are less likely to move great distances. Recapture data for *C. porosus* (Sah and Stuebing, 1996) and *Caiman crocodilus* (Ouboter and Nanhoe, 1998), suggests that juveniles less than 100 cm in length do not move distances of greater than 100 m. It should also be noted that recaptured individuals make up only a very small portion of the population. Furthermore, only minimal movement between sub-populations is needed to maintain panmixis (Wright, 1931).

Among-locality analyses revealed relatively low levels of subdivision. However, the  $R_{ST}$  estimate (0.100) suggests that some subdivision exists, a finding that is not inconsistent with considerable levels of gene flow (Ebert et al., 2002). Mantel's test indicates that  $F_{ST}$  and  $R_{ST}$  values were significantly correlated ( $r = 0.819$ ,  $z = 874$ ,  $pr = 0.01$ ). Thus, both estimates suggest a similar pattern, with the  $R_{ST}$  estimate seeming to provide greater resolution.

Crocodylians are highly mobile on land and have been known to travel considerable distances under certain conditions (Neill, 1971). Platt (1996) reported that adult *C. moreletii* were often observed moving overland, suggesting that these animals do at least occasionally travel from one lagoon to another. Extensive flooding (over 2 m above normal levels), which

results from extreme weather events (e.g., Hurricane Mitch, October 1998; Hurricane Keith, October, 2000) facilitates movement. Such observations are consistent with our findings of gene flow among the seven Morelet's crocodile localities sampled throughout central Belize. We found evidence for some degree of movement of crocodiles between localities. The migration estimate ( $Nm = 5.15$ ) is intermediate when compared to  $Nm$  estimates from lower vertebrate microsatellite studies, including the Timber rattlesnake (*Crotalus horridus*),  $Nm = 0.77-1.9$  (Bushar et al., 1998), Swainson's warbler (*Limnothlypis swainsonii*),  $Nm = 1.5-11.7$  (Winker et al., 2000) and the Piute ground squirrel (*Spermophilus mollis*),  $Nm = 2.3-3.3$  (Van Horne et al., 2001). Overall, the low  $\delta\mu^2$ ,  $F_{ST}$  and  $R_{ST}$  values imply moderate movement of individuals among localities, resulting in little differentiation.

Comparisons of genetic distances and geographic distances appear to support an isolation-by-distance model of gene flow (Table 4). We found the highest differences in those lagoons (specifically Banana Bank Lagoon, which had the highest amount of genetic subdivision in relation to other localities), which are never directly connected to New River-New River Lagoon drainage, even in years of extremely heavy rainfall. Our analyses are consistent with an island model of gene flow (Wright, 1931), where the combined populations of New River and New River Lagoon are acting as a "mainland" and the surrounding smaller populations resemble "islands." Although the heterozygosity levels are not particularly high (Table 1), the large population sizes and considerable number of alleles present in either New River and/or New River Lagoon crocodiles do suggest that this population could be a source of variation for surrounding localities. According to this model, only a small number of migrants originating from this "mainland" are needed to offset the effects of genetic drift. The levels of observed

heterozygosity from smaller populations support this. For example, although the smaller sized Banana Bank Lagoon was inhabited by a relatively small number of crocodiles, the overall heterozygosity estimate ( $H = 0.41$ ) is indicative of a reasonably diverse population. Without New River/New River Lagoon animals serving as a reservoir of genetic variability, such small populations might ultimately face the potentially harmful consequences of inbreeding. It appears that preservation of these “genetic reservoirs” is critical to the overall health of *C. moreletii*.

The Pritchard et al. (2000) analysis (Table 6) appears in large part consistent with this assessment. New River and New River Lagoon animals have microsatellite phenotype patterns that could have been derived from three of the four populations, which is consistent with the hypothesis that they form an important source for genetic variation for smaller populations throughout the region. Only the Banana Bank Lagoon crocodiles are disproportionately ascribed to any single unique population (1), possibly reflecting affiliation to other (as of now uncharacterized) genetic reservoirs from the west. Additional studies directed at addressing this possibility are currently in progress (Ray et al., unpubl.). The other collection localities display similarities in various combinations with the New River/New River Lagoon populations. However, as suggested by the neighbor-joining analysis (Fig. 3), there is little resolution among five of the seven collection localities. Only Gold Button Lagoon and Banana Bank Lagoon, which appear to have immigrants from three of the other, inferred populations (2-4), were resolved clearly. The remaining localities could not be differentiated.

Evolutionary implications.- Microsatellite loci clearly revealed higher levels of variation than those based on protein variation. The magnitudes of the estimates of genetic variability from this

study (e.g.  $F_{ST}$ ,  $R_{ST}$ , allelic diversity, average heterozygosity), are not necessarily inconsistent with Glenn et al.'s (1998) hypothesis that many crocodylian species may have experienced a "pre-historical population bottleneck". If there is a lack of polymorphism observed at loci with lower mutation rates (e.g., isozymes or allozymes) versus loci that have higher mutation rates (e.g., microsatellites), such bottlenecks might still have occurred. However, the relative effects of human activities (such as the hunting of crocodiles or destruction of crocodile habitat) on variation detectable using microsatellite loci are not clear. For the entire family Crocodylidae, both protein analyses (including albumin immunodiffusion data and isozyme phenotypes) and mitochondrial DNA analyses suggest that low levels of genetic divergence frequently exist between species (Densmore, 1983; Densmore and Owen, 1989; Densmore and White, 1991). This may suggest a decrease in the rate of molecular evolution, however, Janke and Arnason (1997) found no evidence of a lower mutation rate in alligators as compared to other vertebrate species. It may also suggest a recent divergence for the Crocodylidae; indeed both molecular (Densmore, 1983) and morphological (Brochu, 2001) evidence support a recent radiation for living members of the genus *Crocodylus*. A recent divergence (perhaps even Pliocene to Pleistocene) along with comparable environmental conditions that could contribute to similar selective pressures on "house-keeping gene" products (e.g., glycolytic or TCA enzymes that are routinely analyzed in allozyme studies) could explain this seeming absence of protein variation across the entire genus. However, additional neutral loci must be examined to test this hypothesis.

The results of this study, which is the first attempt to assess genetic variation using microsatellite analyses in any true crocodile populations, have allowed us to erect a hypothesis

accounting for the way that gene flow occurs between localities inhabited by Morelet's crocodile in north-central Belize. We now need to expand our scope of sampling to include the remainder of Belize and the Yucatan Peninsula to determine whether the patterns of variation and migration we have observed to date are similar across the species range. In addition, now that we have microsatellite marker loci that allow us to more accurately estimate genetic variation, we are also examining other aspects of population and reproductive biology such as the possibility that multiple paternity exists in this species.

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## Figure Legends

**Figure 1** Map of Belize with seven *Crocodylus moreletii* localities and codes. Indian Creek (IC), Habenero Lagoon (HAB), Cox Lagoon (COX), Banana Bank Lagoon (BB), New River (NR), New River Lagoon (NRL), Gold Button Lagoon (GBL).

**Figure 2** Map of the Belize *Crocodylus moreletii* localities, upon which have been superimposed the four populations indicated by model-based clustering of Pritchard et al. (2000). See text for discussion. Locality codes as listed in Figure 1.

**Figure 3** Neighboring-joining tree based on the proportions of individuals at each locality inferred to represent the four underlying populations (Table 6).

Table 1 – separate file due to page formatting problems (TABLE 1)

**Table 2** Genetic structure of localities of *Crocodylus moreletii*, with bootstrapped 95% confidence intervals.

Locus	Estimate	F <sub>IS</sub>		Estimate	F <sub>ST</sub>	
		Lower bound	Upper bound		Lower bound	Upper bound
<i>Cj20</i>	0.017	-0.074	0.085	0.049	0.037	0.096
<i>Cj35</i>	-0.328	-0.414	-0.262	0.017	0.006	0.058
<i>Cj109</i>	0.179	0.066	0.266	0.076	0.048	0.143
<i>Cj119</i>	-0.188	-0.262	-0.13	0.048	0.031	0.105
<i>Cj127</i>	0.131	0.022	0.211	0.116	0.082	0.182
<i>Cj128</i>	-0.019	-0.227	0.184	0.109	0.049	0.212
<i>Cj131</i>	0.172	0.012	0.299	0.068	0.012	0.248
Overall	-0.098	-0.146	-0.076	0.062	0.058	0.092

**Table 3** Mark-recapture information for *Crocodylus moreletii* individuals (ID), distance in meters (D) from original capture spot, time (t) from original capture, sex (S) of individual and total length in cm (TL) of individual .

<u>New River</u>					<u>New River Lagoon</u>				
ID	D	t	S	TL	ID	D	t	S	TL
2544	60	1yr	M	185.3♦	2463	30	1yr	M	135.8
2564	700	1yr	M	175.5♦	2479	450	2mo	M	95.4
2779	0	1yr	M	171.5♦	2829	0	3d	M	48.5
2465	0	1yr	M	119.0	3485	30	5d	M	44.5
2529	10	1yr	M	61.1	2527	1000	1yr	F	136.6
3478	0	4d	M		2524	80	1yr	F	136.0
3474	40	3d	M		2497	0	4d	F	82.8
3439	10	16d	M	57.0					
Mean	102.5			128.23		227			97

Distance measures were calculated from GPS coordinates. (♦) indicates sexually mature individuals.

**Table 4** Pairwise comparison of the genetic structure of seven populations of *Crocodylus moreletii* in Belize. Above diagonal, pairwise  $F_{ST}$  values, below diagonal, pairwise  $R_{ST}$  values. Localities presented in order of relative geographic distance from the New River Lagoon.

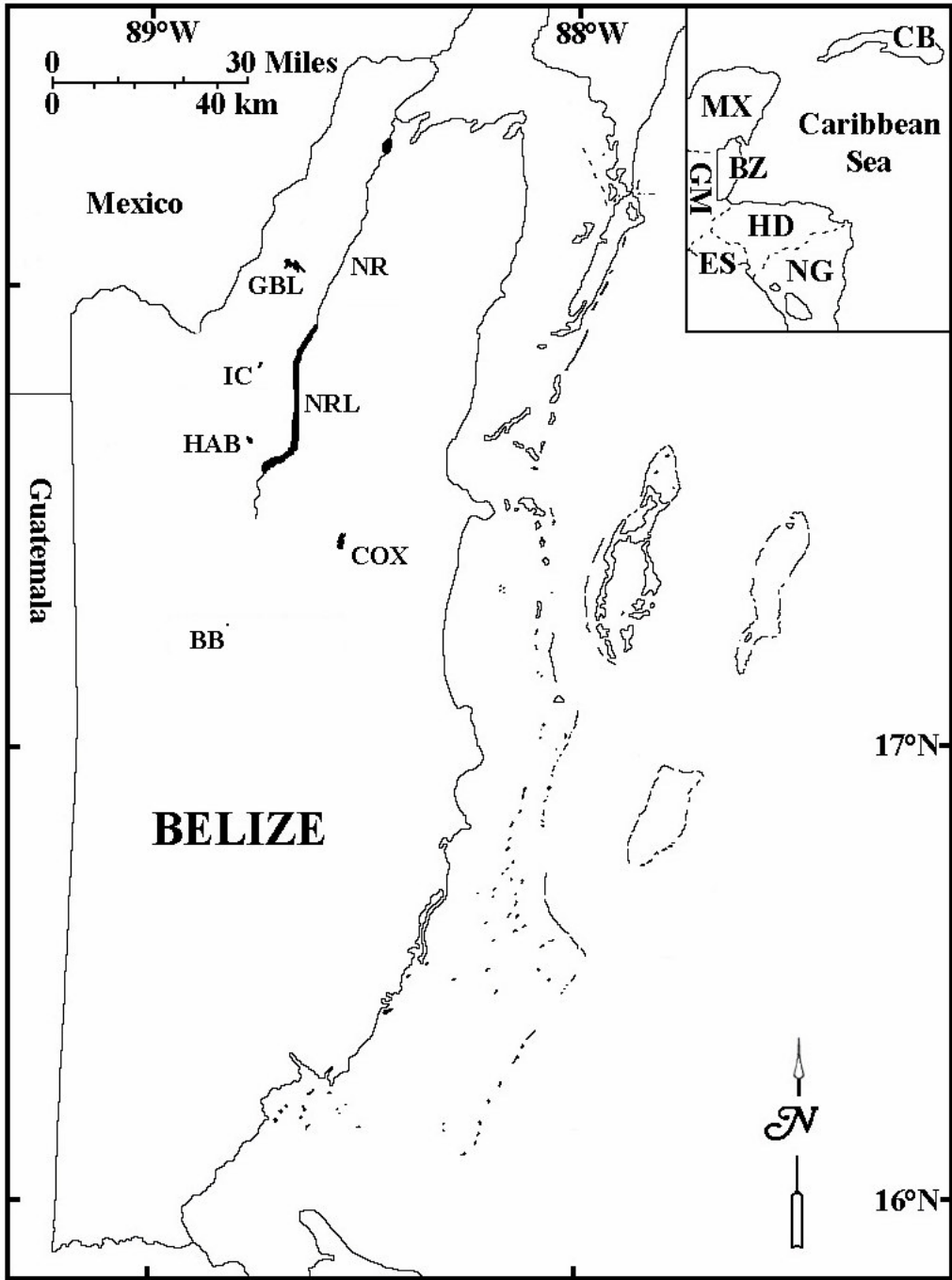
Locality	New River Lagoon	New River	Indian Creek	Habenero Lagoon	Gold Button Lagoon	Cox Lagoon	Banana Bank
New River Lagoon	-----	<b>0.011</b>	<b>-0.005</b>	<b>0.051</b>	<b>0.039</b>	<b>0.111</b>	<b>0.149</b>
New River	<b>0.008</b>	-----	0.029	0.051	0.036	0.114	0.152
Indian Creek	<b>0.057</b>	-0.074	-----	0.109	0.058	0.070	0.097
Habenero Lagoon	<b>0.050</b>	0.021	0.069	-----	0.103	0.195	0.226
Gold Button Lagoon	<b>0.033</b>	0.025	-0.029	0.070	-----	0.126	0.233
Cox Lagoon	<b>0.180</b>	0.061	0.013	0.194	0.133	-----	0.199
Banana Bank	<b>0.393</b>	0.276	0.369	0.369	0.371	0.158	-----

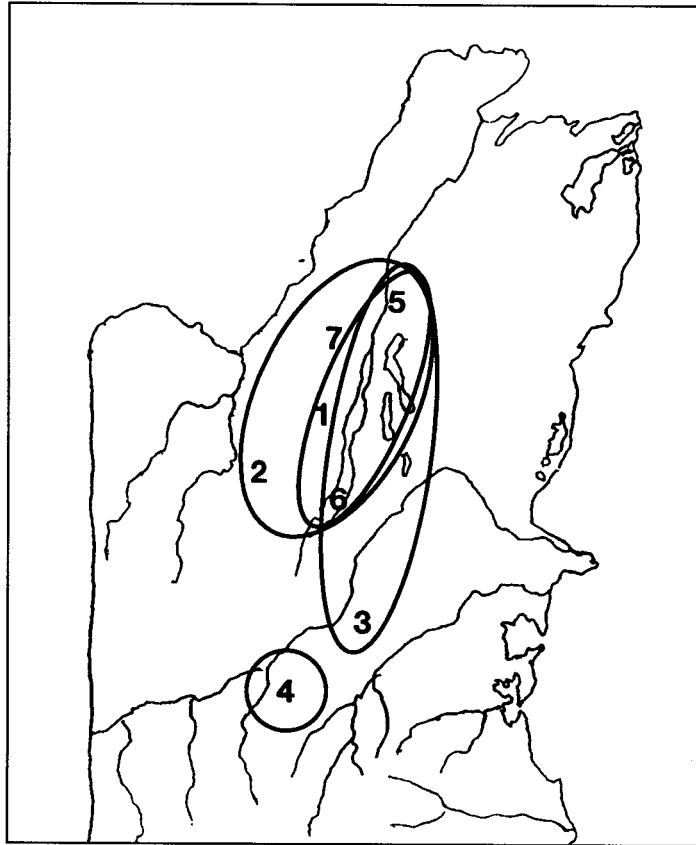
**Table 5** Posterior probabilities associated with varying numbers of hypothetical underlying populations (K) from the Pritchard et al. (2000) analysis.  $\Pr(X|K)$  is the estimated probability of the observed data, given a hypothetical number of populations;  $\Pr(K)$  is the resulting estimate of the posterior probability of that number of populations.

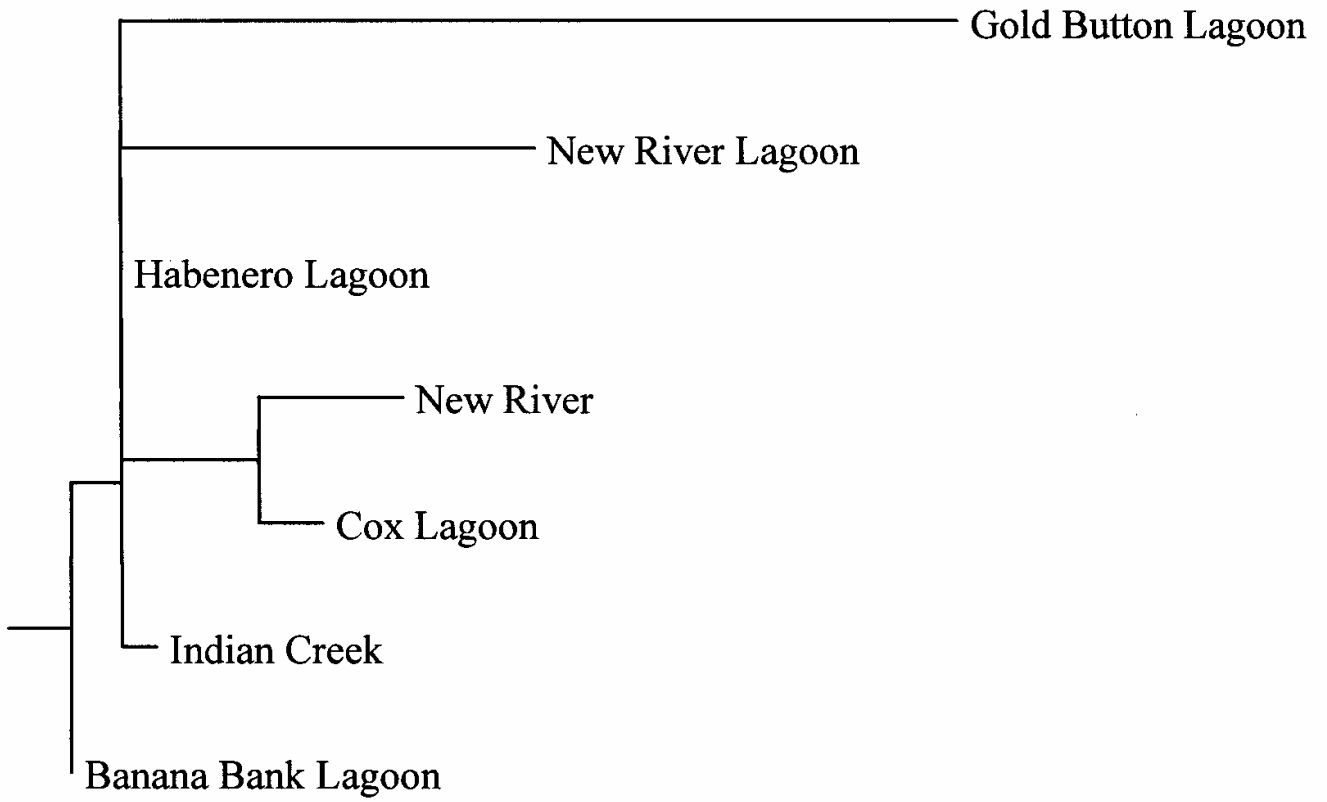
K	$\ln [\Pr(X K)]$	$\Pr(K)$
1	-3669	~0
2	-3611	~0
3	-3586	~0
4	-3574	~1
5	-3654	~0
6	-3704	~0
7	-3739	~0

**Table 6** Probabilities of membership of individuals at each locality assigned to each of the four inferred underlying populations as determined by Pritchard et al. (2000) analysis (see text for details).

Locality	1	2	3	4
Indian Creek	0.110	0.337	0.058	0.495
Habenero	0.108	0.228	0.052	0.611
Cox	0.061	0.135	0.633	0.170
Banana Bank	0.825	0.073	0.059	0.043
New River	0.077	0.296	0.234	0.393
New River Lagoon	0.063	0.371	0.203	0.364
Gold Button	0.040	0.176	0.320	0.464







**Appendix I** Allele frequencies and standard errors (SE) for 9 microsatellite loci, from Morelet's crocodile populations. Allele numbers correspond to base-pair size from ABI Genescan ladder.

Loc	Locus	Allele	Count	Frequency	SE	Loc	Locus	Allele	Count	Frequency	SE
NR	<i>Cj128</i>	205	3	0.016	0.009	NRL	<i>Cj128</i>	221	2	0.015	0.01
		221	1	0.005	0.005			223	90	0.662	0.041
		223	126	0.692	0.034			228	1	0.007	0.007
		224	1	0.005	0.005			229	32	0.235	0.036
		229	30	0.165	0.028			233	6	0.044	0.018
		235	2	0.011	0.008			235	1	0.007	0.007
		236	1	0.005	0.005			245	2	0.015	0.01
		245	1	0.005	0.005			253	2	0.015	0.01
		246	3	0.016	0.009		<i>Cj18</i>	190	66	0.485	0.043
		253	8	0.044	0.015			191	68	0.5	0.043
		254	1	0.005	0.005			192	2	0.015	0.01
		255	5	0.027	0.012		<i>Cj20</i>	149	17	0.127	0.029
	<i>Cj18</i>	190	73	0.397	0.036			173	17	0.127	0.029
		191	107	0.582	0.036			175	75	0.56	0.043
		192	3	0.016	0.009			177	18	0.134	0.029
		200	1	0.005	0.005			179	7	0.052	0.019
	<i>Cj20</i>	149	12	0.069	0.019		<i>Cj35</i>	134	2	0.015	0.011
		171	1	0.006	0.006			144	39	0.295	0.04
		173	39	0.224	0.032			146	89	0.674	0.041
		175	83	0.477	0.038			165	2	0.015	0.011
		177	28	0.161	0.028		<i>Cj109</i>	360	2	0.015	0.01
		179	11	0.063	0.018			362	53	0.396	0.042
	<i>Cj35</i>	144	32	0.184	0.029			365	18	0.134	0.029
		146	138	0.793	0.031			367	2	0.015	0.01
		165	4	0.023	0.011			368	1	0.007	0.007
	<i>Cj109</i>	346	1	0.006	0.006			371	1	0.007	0.007
		362	92	0.561	0.039			373	52	0.388	0.042
		365	13	0.079	0.021			377	4	0.03	0.015
		367	2	0.012	0.009			380	1	0.007	0.007
		370	1	0.006	0.006		<i>Cj119</i>	175	5	0.038	0.017
		373	54	0.329	0.037			177	19	0.144	0.031
		377	1	0.006	0.006			179	108	0.818	0.034
	<i>Cj119</i>	175	4	0.022	0.011		<i>Cj127</i>	336	69	0.507	0.043
		177	32	0.174	0.028			338	9	0.066	0.021
		179	148	0.804	0.029			344	6	0.044	0.018
	<i>Cj127</i>	334	2	0.011	0.008			353	44	0.324	0.04
		336	92	0.511	0.037			355	7	0.051	0.019

Loc	Locus	Allele	Count	Frequency	SE	Loc	Locus	Allele	Count	Frequency	SE
		338	18	0.1	0.022			377	1	0.007	0.007
		344	10	0.056	0.017		<i>Cj16</i>	132	136	1	0
		351	1	0.006	0.006		<i>Cj131</i>	208	1	0.007	0.007
		351	1	0.006	0.006		<i>Cj131</i>	208	1	0.007	0.007
		353	36	0.2	0.03			210	124	0.912	0.024
		355	18	0.1	0.022			211	3	0.022	0.013
		376	1	0.006	0.006			217	4	0.029	0.014
		377	1	0.006	0.006			218	3	0.022	0.013
		378	1	0.006	0.006			228	1	0.007	0.007
	<i>Cj16</i>	132	167	0.938	0.018	HAB	<i>Cj128</i>	223	22	1	0
		152	10	0.056	0.017		<i>Cj18</i>	190	9	0.409	0.105
		273	1	0.006	0.006			191	13	0.591	0.105
	<i>Cj131</i>	210	172	0.935	0.018		<i>Cj20</i>	175	7	0.318	0.099
		217	5	0.027	0.012			176	2	0.091	0.061
		218	7	0.038	0.014			177	13	0.591	0.105
GBL	<i>Cj128</i>	223	57	0.713	0.051		<i>Cj35</i>	144	6	0.273	0.095
		227	2	0.025	0.017			146	16	0.727	0.095
		229	16	0.2	0.045		<i>Cj109</i>	362	13	0.591	0.105
		239	2	0.025	0.017			371	2	0.091	0.061
		253	3	0.037	0.021			373	7	0.318	0.099
	<i>Cj18</i>	190	42	0.525	0.056		<i>Cj119</i>	179	22	1	0
		191	36	0.45	0.056		<i>Cj127</i>	336	11	0.5	0.107
		199	1	0.013	0.012			353	2	0.091	0.061
		200	1	0.013	0.012			355	7	0.318	0.099
	<i>Cj20</i>	149	11	0.138	0.039			357	1	0.045	0.044
		153	3	0.037	0.021			359	1	0.045	0.044
		173	23	0.287	0.051		<i>Cj16</i>	132	20	1	0
		175	31	0.388	0.054		<i>Cj131</i>	210	21	0.955	0.044
		177	12	0.15	0.04	BB	<i>Cj128</i>	223	12	0.4	0.089
	<i>Cj35</i>	144	24	0.3	0.051			245	5	0.167	0.068
		146	56	0.7	0.051			253	12	0.4	0.089
	<i>Cj109</i>	362	54	0.75	0.051			255	1	0.033	0.033
		365	2	0.028	0.019		<i>Cj18</i>	190	12	0.4	0.089
		371	2	0.028	0.019			191	18	0.6	0.089
		373	13	0.181	0.045		<i>Cj20</i>	175	19	0.679	0.088
		379	1	0.014	0.014			177	1	0.036	0.035
	<i>Cj119</i>	175	7	0.087	0.032			179	1	0.036	0.035
		177	12	0.15	0.04			197	7	0.25	0.082
		179	61	0.762	0.048		<i>Cj35</i>	144	5	0.192	0.077

Loc	Locus	Allele	Count	Frequency	SE	Loc	Locus	Allele	Count	Frequency	SE
	<i>Cj127</i>	332	1	0.013	0.012			146	21	0.808	0.077
		334	1	0.013	0.012		<i>Cj109</i>	362	2	0.083	0.056
		336	26	0.325	0.052			365	5	0.208	0.083
		338	4	0.05	0.024			373	17	0.708	0.093
		344	1	0.013	0.012		<i>Cj119</i>	177	12	0.4	0.089
		353	46	0.575	0.055			179	18	0.6	0.089
		355	1	0.013	0.012		<i>Cj127</i>	336	5	0.167	0.068
	<i>Cj128</i>	132	71	0.934	0.028			338	2	0.067	0.046
		150	2	0.026	0.018			355	22	0.733	0.081
		154	3	0.039	0.022			357	1	0.033	0.033
	<i>Cj131</i>	210	78	0.975	0.017		<i>Cj16</i>	132	28	1	0
		218	1	0.013	0.012		<i>Cj131</i>	210	26	0.867	0.062
		224	1	0.013	0.012			220	4	0.133	0.062
IC	<i>Cj128</i>	223	5	0.833	0.152	COX	<i>Cj128</i>	223	4	0.4	NA
		229	1	0.167	0.152			229	4	0.4	
	<i>Cj18</i>	190	2	0.333	0.192			245	1	0.1	
		191	4	0.667	0.192			253	1	0.1	
	<i>Cj20</i>	149	1	0.167	0.152		<i>Cj18</i>	190	4	0.4	
		175	4	0.667	0.192			191	6	0.6	
		179	1	0.167	0.152		<i>Cj20</i>	173	1	0.1	
	<i>Cj35</i>	144	1	0.25	0.217			175	5	0.5	
		146	3	0.75	0.217			177	4	0.4	
	<i>Cj109</i>	362	3	0.5	0.204		<i>Cj35</i>	144	5	0.5	
		373	3	0.5	0.204			146	5	0.5	
	<i>Cj119</i>	177	3	0.5	0.204		<i>Cj109</i>	362	4	0.4	
		179	3	0.5	0.204			365	1	0.1	
	<i>Cj127</i>	336	4	0.667	0.192			370	1	0.1	
		353	1	0.167	0.152			371	2	0.2	
		355	1	0.167	0.152			373	1	0.1	
	<i>Cj16</i>	132	5	0.833	0.152			377	1	0.1	
		152	1	0.167	0.152		<i>Cj119</i>	175	2	0.2	
	<i>Cj131</i>	210	6	1	0			177	3	0.3	
								179	5	0.5	
							<i>Cj127</i>	336	2	0.2	
								338	2	0.2	
								344	2	0.2	
								353	3	0.3	
								378	1	0.1	

Loc	Locus	Allele	Count	Frequency	SE	Loc	Locus	Allele	Count	Frequency	SE
	COX	<i>Cj128</i>	223	4	0.4						
			229	4	0.4						
			245	1	0.1						
			253	1	0.1						
		<i>Cj131</i>	210	5	0.5						
			217	1	0.1						
			218	4	0.4						