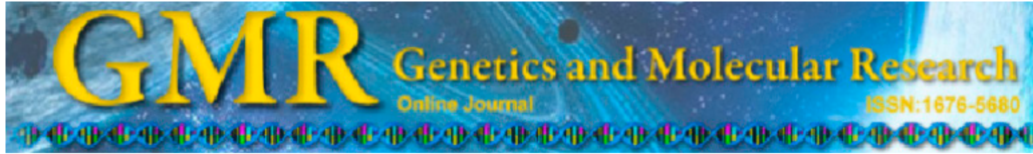

Assessment of microsatellites in estimating inter- and intraspecific variation among Neotropical *Crocodylus* species

A. Bashyal[1]*, B.A. Gross[1]*, M. Venegas-Anaya[1,2], F. Lowrance[1] and L.D. Densmore III[1]

[1]Department of Biological Sciences, Texas Tech University, Lubbock, TX, USA
[2]Smithsonian Tropical Research Institute, Balboa, Ancón, Republic of Panama

*These authors contributed equally to this study.
Corresponding author: M. Venegas-Anaya
E-mail: dracocodrilo@hotmail.com

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ABSTRACT. We tested microsatellites that were developed for the saltwater crocodile (*Crocodylus porosus*) for cross-species amplification and to provide an estimate of inter- and intraspecific variation among four species of Neotropical crocodiles (*C. rhombifer*, *C. intermedius*, *C. acutus*, and *C. moreletii*). Our results indicated that with the exception of 2 loci in *C. intermedius*, all 10 microsatellite loci were successfully amplified in the 4 species, producing a set of variably sized alleles that ranged in number between 2 and 14 alleles per locus. Similarly, private alleles (i.e., unique alleles) also were reported in all 4 species for at least 3 loci. The mean observed and expected heterozygosities (averaged across species for all 10 loci combined) ranged from 0.39 to 0.77 and from 0.44 to 0.78, respectively. In addition to this, we evaluated these microsatellites in 2 populations of *C. acutus* and *C. moreletii* to assess their utility in estimating intraspecific levels of polymorphisms. These

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microsatellites also showed considerable allelic variation in population level analysis. The set of 10 microsatellite loci in our study had the potential to be used as a tool in population and conservation genetic studies of Neotropical crocodiles.

Key words: Microsatellites; Crocodiles; Cross-species amplification; Neotropical crocodiles

INTRODUCTION

Microsatellites are repetitive sequences of 1 to 6 bp of DNA that are mostly found in the non-coding regions of the eukaryotic genome and are known to have high mutation rates that lead to greater allelic variability and high levels of polymorphism (Wright and Bentzen, 1994). Because they are hypervariable and occur ubiquitously, microsatellites have widely been used as tools for a variety of fields of study such as population and conservation genetics, molecular ecology (mating behavior and gene flow), and wildlife DNA forensic analyses (Glenn et al., 1998; Fitzsimmons et al., 2001; Davis et al., 2001, 2002; Dever et al., 2002; Avise, 2004; Anmarkrud et al., 2008).

Despite their widespread application in this discipline, one drawback of using microsatellites as genetic markers has been the development of primers, which can involve both considerable time and resources. Fortunately, a number of microsatellite loci are conserved enough across species to be useful in interspecific and intraspecific marker comparisons (Glenn et al., 1998). Successful cross-species amplification of microsatellites has been reported from groups such as mammals (Williamson et al., 2002; Gunn et al., 2005), birds (Primmer et al., 1996, 2005), insects (Wilson et al., 2004), fish (Scribner et al., 1996; King et al., 2001), snakes (King, 2009; Vandewege et al., 2012), and crocodilians (Dever and Densmore III, 2001; Zucoloto et al., 2006; Weaver et al., 2008; Milián-Garcia et al., 2011; Rodriguez et al., 2008, 2011).

The "Neotropical crocodiles" consist of 4 species: the American crocodile (*Crocodylus acutus*), Cuban crocodile (*C. rhombifer*), Orinoco crocodile (*C. intermedius*), and Morelet's crocodile (*C. moreletii*). Of the 4 species, 2 (*C. rhombifer* and *C. intermedius*) are considered critically endangered (Crocodile Specialist Group, 1996; Targarona et al., 1996), whereas *C. acutus* is considered vulnerable (Ponce-Campos et al., 2012) and *C. moreletii* is regarded as lower concern (Cedeño-Vázquez et al., 2012). Given their conservation status, having a larger number of successfully amplified microsatellite loci that exhibit high levels of polymorphism at the inter- and intraspecific levels in these Neotropical crocodiles could serve as an important conservation tool.

Recently, Miles et al. (2009a) developed 253 novel microsatellite loci from the saltwater crocodile (*C. porosus*) library and tested 82 microsatellites for cross-species amplification in 18 species of crocodilians (Miles et al., 2009b). The set of microsatellites included in our study consisted of 5 loci that were successfully cross-species amplified by Miles et al. (2009b) and 5 loci tested herein for the first time for cross-species amplification in Neotropical crocodiles from Miles et al. (2009a).

We evaluated these 10 microsatellite loci for cross-species amplification in all 4 species of Neotropical crocodiles and in populations from 2 of these species, *C. acutus* (from

Panama and Mexico) and *C. moreletii* (from Mexico and Belize). The objectives of our study were to: a) test microsatellite primers that were developed for *C. porosus* for cross-species amplification in Neotropical crocodiles, b) assess the utility of these microsatellites in estimating levels of inter- and intraspecific polymorphism among the Neotropical crocodiles, and c) identify private alleles (alleles that are unique to a particular species or population) at the species and population levels that can be used to identify species and populations.

MATERIAL AND METHODS

Tissue samples were taken from 5 *C. rhombifer*, 5 *C. intermedius*, 26 *C. acutus* (16 from Panama and 10 from Mexico), and 45 *C. moreletii* (32 from Belize and 13 from Mexico) individuals. Total genomic DNA was isolated by proteinase K digestion and extracted using the cetyltrimethylammonium bromide-phenol-chloroform technique (Sambrook et al., 1989; Palumbi, 1996). We first chose 40 microsatellite loci from Miles et al. (2009a) that were reported to have high levels of polymorphism. We then tested those 40 loci for cross-species amplification in *C. moreletii* and *C. acutus*, and only 10 loci showed consistent amplification. The 10 microsatellite loci that showed cross-species amplification in *C. moreletii* and *C. acutus* were further tested for cross-species amplification in other Neotropical crocodiles using a 2-primer polymerase chain reaction (PCR). Sequence-specific forward primers from Miles et al. (2009a) were modified by adding a 5'-M13 tail, whereas the reverse primers were used as they were designed. The M13 tail is a complementary oligonucleotide sequence of a universal M13 fluorescence nucleic acid polymer (5'-CACGACGTTGTAAAACGAC-3'). The final amplification was performed using "3-primer competition" PCR (Schuelke, 2000) (Tables 1 and 2). Fluorescent dye labels (FAM, VIC, NED, and PET; Applied Biosystems, USA) were used to label the universal M13 nucleotide 5'-tail (Table 1). The PCR products were electrophoresed on 2% agarose gels and run for 20 min at 70-75 V.

PCRs were carried out using 2 different programs (Table 1). The TD-65 program (modified from Miles et al., 2009a) consisted of an initial denaturation step of 95°C for 3 min; 4 cycles of 95°C for 30 s, 65°C for 30 s, and 72°C for 45 s; 4 cycles of 95°C for 30 s, 62°C for 30 s, and 72°C for 45 s; 8 cycles of 95°C for 30 s, 60°C for 30 s, and 72°C for 45 s; 24 cycles of 95°C for 30 s, 55°C for 30 s, and 72°C for 45 s; and a final extension step at 72°C for 5 min. Similarly, the T-55 program consisted of an initial denaturation step of 94°C for 3 min; 34 cycles of 94°C for 40 s, 55°C for 40 s, and 72°C for 60 s; and a final extension step at 72°C for 5 min. An ABI 3100 Avant genetic analyzer (Applied Biosystems) was used to detect and size fragments that were fluorescently labeled with the reaction (for each microsatellite), which consisted of a final volume of 12 µL including 0.6 µL LIZ (500) standard, 1.5 µL PCR product, and 9.9 µL formamide.

Electrophoretograms were analyzed in GENEMAPPER 3.7 (Applied Biosystems) using the following analysis method: the size calling parameter was set at local Southern, the signal levels for homozygous and heterozygous minimum peak height were set at 140 and 85, respectively (following the manufacturer guideline for ABI 3100), and the minimum peak height ratio was set at 0.2. These parameters were selected to identify any stutter peaks that might be present and also to identify the alleles that might have unequal amplification and thus were not scored. During the amplification process of microsatellites, the formation of stutter peaks is usually caused by the effects of polymerase slippage, which can ultimately cause a single or multiple repeat units to be skipped or added to the strands that are produced during

Table 1. Primer sequences (provided by Miles et al., 2009a), fluorescent dye labels, and PCR conditions for the 10 microsatellite loci.

Locus	Primers (5' to 3')	Fluorescent dye label	PCR conditions
CpDi06	F: TGTTGGGCACTTTGAAC R: GTTTAAGAAAAATGGTGGAAAAC	PET	T-55
CpP2201	F: CAAGTGACCCCTTTTCAG R: GTTTATTTCTTGTTTGGCACATC	VIC	T-55
CpP2202*	F: GCAACAAAAGACCTTGACA R: GTTTGGTTGGGTGGAATTATATAC	NED	T-55
CpP208*	F: CACATGGCTTTTGTTCTGAG R: GTTTCCTGCAAAATGTTCTCCTA	FAM	TD-65
CpP1306*	F: TTCTCTCTAGGAGCCACTCAC R: GTTTAGGGAGACATCTAGGAAGAAC	NED	TD-65
CpP801	F: TTGGCATTAGATTGGTAGAC R: GTTTCTATGCCAAAGCTACAAC	VIC	T-55
CpDi04*	F: TTCTAAACAGTCCAGGATGA R: GTTTAAATTTCACTAGATGCCATAA	FAM	T-55
CpP815	F: GGTTAAGTGCCACACAAGT R: GTTTGGCCAATTTCTAATGAA	NED	T-55
CpP314*	F: GAAATGCCACTAATACACACA R: GTTCCAATTCTTCAGGTCCTTAT	FAM	T-55
CpP4311	F: GGCTGCTCTGTGTTTG R: GTTTGGGTTTAGCATCATGT	VIC	TD-65
CpDi06	F: TGTTGGGCACTTTGAAC R: GTTTAAGAAAAATGGTGGAAAAC	PET	T-55
CpP2201	F: CAAGTGACCCCTTTTCAG R: GTTTATTTCTTGTTTGGCACATC	VIC	T-55
CpP2202*	F: GCAACAAAAGACCTTGACA R: GTTTGGTTGGGTGGAATTATATAC	NED	T-55
CpP208*	F: CACATGGCTTTTGTTCTGAG R: GTTTCCTGCAAAATGTTCTCCTA	FAM	TD-65
CpP1306*	F: TTCTCTCTAGGAGCCACTCAC R: GTTTAGGGAGACATCTAGGAAGAAC	NED	TD-65
CpP801	F: TTGGCATTAGATTGGTAGAC R: GTTTCTATGCCAAAGCTACAAC	VIC	T-55
CpDi04*	F: TTCTAAACAGTCCAGGATGA R: GTTTAAATTTCACTAGATGCCATAA	FAM	T-55
CpP815	F: GGTTAAGTGCCACACAAGT R: GTTTGGCCAATTTCTAATGAA	NED	T-55
CpP314*	F: GAAATGCCACTAATACACACA R: GTTCCAATTCTTCAGGTCCTTAT	FAM	T-55
CpP4311	F: GGCTGCTCTGTGTTTG R: GTTTGGGTTTAGCATCATGT	VIC	TD-65

*Previously untested microsatellites from Miles et al. (2009a).

Table 2. Reaction mix for amplification of the 10 microsatellite loci.

Reagents	Final concentration of each reagent for the respective loci	
	CpDi06, CpP2201, CpP2202, CpP208	CpP1306, CpP801, CpDi04, CpP815, CpP314 , CpP4311
Taq buffer	1X	1X
$MgCl_2$	2 mM	2 mM
dNTPs	0.52 mM each	0.52 mM each
Forward primer	0.2 µM	0.2 µM
Reverse primer	0.2 µM	0.2 µM
M13	0.1 µM	0.1 µM
Taq Polymerase*	0.03 U/µL	0.028 U/µL
Template DNA	1 ng/µL	0.8 ng/µL

*Qiagen Taq polymerase.

PCR (Watzinger et al., 2006). Using the fluorescent-based PCR technique, stutter peaks can be recognized as additional products of the PCR that have a lower peak height; the majority of the time, they tend to appear 1 repeat unit downstream from the true allele (Watzinger et al., 2006).

CERVUS 3.0 (Marshall et al., 1998) was used to estimate the number of alleles (*A*), observed heterozygosity (H_O), and expected heterozygosity (H_E). GENALEX 6.0 (Peakall and Smouse, 2006) was used to estimate the allele frequency, number of private alleles (A_p), and probability of identity (*PI*) by locus.

RESULTS

Cross-species amplification was observed among all possible PCRs involving *C. acutus*, *C. moreletii*, and *C. rhombifer*, and it was observed at the 80% level for *C. intermedius*. With only 2 exceptions (CpDi06 and CpP801 for *C. intermedius*), all 10 microsatellite loci were successfully amplified in all 4 species of Neotropical crocodiles. All amplified loci produced a variety of alleles and appeared to be polymorphic in all 4 species with the exception of 1 locus (CpP2202) in *C. rhombifer*, which was found to be monomorphic.

Stutter peaks of all 10 loci were present in low frequencies for one or more species (Table 3). The presence of stutter peaks can potentially create incorrect scoring between alleles, where size differences are small, and can lead to calling true-heterozygotes as pseudo-homozygotes or the opposite (Skotheim et al., 2001). Using an appropriate analysis method in GENEMAPPER 3.7 (see above in Material and Methods) to analyze the electrophoretograms, we were able to identify stutter peaks and any other alleles that were not scored due to unequal amplification, which reduced the misidentification of alleles.

A total of 94 different alleles were found among the 10 loci combined for all species. The *A* value ranged from 2 to 14 with an overall mean of 4.95 (Table 3). The estimates of H_O and H_E ranged from 0.39 to 0.77 and 0.44 to 0.78, respectively, with a mean of 0.62 and 0.61, respectively (Table 3). The *PI* by locus (the probability that 2 individuals drawn at random from a population will have the same genotype at multiple loci) ranged from 0.08 to 0.38 with a mean of 0.18 (Table 3). Diagnostic private (unique) alleles were found in all 4 species for at least 3 loci (Table 3). One interesting observation was that, although only 5 individuals of *C. rhombifer* were genotyped, there were a total of 33 alleles and 8 private alleles reported for this species, further demonstrating the hyper-variability of these microsatellites.

A finer scale population-level analyses (Tables 4 and 5) showed that all the microsatellite loci were polymorphic and that there was considerable allelic variation between the 2 populations of *C. acutus* from Panama (mean 4.4 alleles per locus) and Mexico (mean 4.8 alleles per locus), as well as between the 2 populations of *C. moreletii* from Mexico (mean of 4 alleles per locus) and Belize (mean 6.6 alleles per locus). Private alleles were reported in each population for at least 3 loci (Table 4). Although the frequencies of the private alleles were generally low, 2 alleles (allele 242 of locus CpDi06 and allele 149 of locus CpP801) in the *C. acutus* population from Panama had reasonably high frequencies (0.47 for both; Table 5). The mean H_O was higher in the population of *C. acutus* from Mexico (0.70) than that from Panama (0.61; Table 4); however, there was not a statistically significant difference between the mean H_O of these 2 populations ($P > 0.05$). Similarly, the mean H_O was higher in the population of *C. moreletii* from Belize (0.76) than that from Mexico (0.70; Table 4); however, there was not a statistically significant difference between the mean H_O of these 2 populations ($P > 0.05$).

Table 3. Number of alleles per locus (A), number of private alleles per locus (A_P), probability of identity (PI), size range of the PCR products, observed heterozygosity (H_O), and expected heterozygosity (H_E) of 10 microsatellite loci in each of the 4 species of Neotropical crocodiles.

Locus	Species	A	A_P	PI	Size (bp)	H_O	H_E
CpP2201	*C. rhombifer**	3	1	0.08	183-235	0.2	0.64
	C. intermedius	4	1	0.26	179-227	0.4	0.53
	C. acutus	6	1	0.08	191-235	1	0.73
	C. moreletii	4	0	0.18	191-227	0.86	0.57
	Mean	4.25	-	0.15	-	0.62	0.62
		A	A_P	PI	Size (bp)	H_O	H_E
	C. rhombifer	1	0	0.39	184	-	-
CpP2202	*C. intermedius**	3	1	0.38	180-196	0.6	0.69
	C. acutus	2	0	0.18	188-200	0.96	0.51
	C. moreletii	3	0	0.18	188-200	0.96	0.58
	Mean	2.25	-	0.28	-	0.63	0.45
		A	A_P	PI	Size (bp)	H_O	H_E
	*C. rhombifer**	3	2	0.05	218-236	0.6	0.51
CpDi06	*C. intermedius*	-	-	-	-	-	-
	C. acutus	10	7	0.18	224-264	0.92	0.76
	C. moreletii	5	1	0.18	226-248	0.77	0.55
	Mean	4.5	-	0.10	-	0.57	0.46
		A	A_P	PI	Size (bp)	H_O	H_E
	*C. rhombifer**	3	1	0.39	179-191	0.2	0.51
CpP208	*C. intermedius*	2	0	0.38	179-191	0.8	0.53
	*C. acutus**	2	0	0.38	179-191	0.92	0.51
	C. moreletii	2	0	0.38	179-191	0.71	0.46
	Mean	2.25	-	0.38	-	0.66	0.50
		A	A_P	PI	Size (bp)	H_O	H_E
	C. rhombifer	5	2	0.25	234-270	0.6	0.82
CpP314	*C. intermedius*	4	0	0.13	246-266	1	0.78
	C. acutus	8	0	0.09	246-282	0.5	0.80
	C. moreletii	10	4	0.09	238-286	0.64	0.73
	Mean	6.75	-	0.14	-	0.69	0.78
		A	A_P	PI	Size (bp)	H_O	H_E
	C. rhombifer	4	0	0.39	195-211	1	0.73
CpP4311	*C. intermedius**	2	0	0.18	187-199	1	0.56
	C. acutus	4	0	0.14	195-219	0.28	0.54
	C. moreletii	7	2	0.14	175-219	0.58	0.59
	Mean	4.25	-	0.21	-	0.72	0.61
		A	A_P	PI	Size (bp)	H_O	H_E
	C. rhombifer	4	0	0.11	218-242	0.8	0.80
CpP815	*C. intermedius*	2	0	0.08	222-242	1	0.56
	C. acutus	10	4	0.05	206-246	0.46	0.85
	C. moreletii	6	0	0.09	218-246	0.82	0.71
	Mean	5.5	-	0.08	-	0.77	0.73
		A	A_P	PI	Size (bp)	H_O	H_E
	C. rhombifer	2	0	0.22	157-169	0.4	0.36
CpP801	*C. intermedius*	-	-	-	-	-	-
	C. acutus	10	2	0.05	145-185	0.56	0.85
	C. moreletii	14	6	0.03	141-201	0.75	0.86
	Mean	6.5	-	0.08	-	0.43	0.52
		A	A_P	PI	Size (bp)	H_O	H_E
	*C. rhombifer**	5	2	0.23	156-170	0.6	0.87
CpDi04	*C. intermedius**	3	2	0.46	164-176	0.2	0.73
	C. acutus	6	1	0.18	142-166	0.17	0.49
	C. moreletii	8	1	0.14	142-166	0.59	0.64
	Mean	5.5	-	0.25	-	0.39	0.68
		A	A_P	PI	Size (bp)	H_O	H_E
CpP1306	*C. rhombifer*	3	0	0.16	93-109	0.4	0.60
	C. intermedius	6	0	0.11	85-121	1	0.84
	C. acutus	9	1	0.05	81-149	0.63	0.78
	C. moreletii	13	4	0.05	77-153	0.81	0.83
	Mean	7.75	-	0.09	-	0.71	0.76

*Absence of stutter peaks in that species for that particular locus.

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Table 4. Number of alleles per locus (A), number of private alleles per locus (A_P), probability of identity (PI), observed heterozygosity (H_O), and expected heterozygosity (H_E) of 10 microsatellite loci in each of the 2 populations of *Crocodylus acutus* and *C. moreletii*.

	C. acutus - Panama (N = 16)					*C. acutus* - Mexico (N = 10)					*C. moreletii* - Mexico (N = 13)					*C. moreletii* - Belize (N = 32)				
Locus	A	A_P	PI	H_O	H_E	A	A_P	PI	H_O	H_E	A	A_P	PI	H_O	H_E	A	A_P	PI	H_O	H_E
CpP2201	3	1	0.30	1.00	0.57	5	0	0.11	1.00	0.79	3	0	0.28	1.00	0.59	3	0	0.30	0.81	0.56
CpP2202	2	0	0.38	1.00	0.52	2	0	0.38	0.90	0.52	3	0	0.32	1.00	0.56	3	0	0.27	0.94	0.59
CpDi06	2	1	0.38	0.94	0.51	9	5	0.04	0.89	0.90	2	0	0.38	0.75	0.52	5	2	0.27	0.78	0.57
CpP208	2	0	0.38	0.94	0.51	2	0	0.38	0.89	0.52	2	0	0.38	0.92	0.52	2	0	0.42	0.63	0.44
CpP314	6	1	0.22	0.44	0.59	5	0	0.14	0.60	0.72	4	0	0.16	0.54	0.71	10	4	0.11	0.69	0.72
CpP4311	4	0	0.42	0.19	0.38	2	0	0.49	0.44	0.37	4	0	0.46	0.39	0.35	7	3	0.16	0.66	0.66
CpP815	8	3	0.09	0.31	0.78	6	1	0.08	0.70	0.81	4	1	0.16	0.77	0.72	5	0	0.15	0.84	0.70
CpP801	8	2	0.09	0.50	0.76	6	0	0.14	0.67	0.72	8	2	0.06	0.58	0.85	11	3	0.04	0.81	0.86
CpDi04	2	0	0.88	0.06	0.06	6	1	0.16	0.43	0.68	4	0	0.18	0.30	0.69	8	3	0.21	0.68	0.63
CpP1306	7	1	0.12	0.69	0.72	5	0	0.12	0.50	0.77	6	1	0.11	0.80	0.78	12	3	0.06	0.81	0.83
Mean	4.4	-	0.33	0.61	0.54	4.8	-	0.20	0.70	0.68	4.0	-	0.25	0.71	0.63	6.6	-	0.20	0.77	0.66

DISCUSSION

All 10 microsatellites that were tested in our study were informative and produced a variety of both shared and private alleles at both the cross-species and inter-population levels. The number of alleles in a population (allelic richness) and the number of unique alleles in a population (private allelic richness) are considered to be useful for many conservation and population genetics applications (Kalinowski, 2004) and can be used to infer the evolutionary history of a population (Castric and Bernatchez, 2003). Together, these measures of genetic diversity can be beneficial in identifying populations of Neotropical crocodiles that exhibit low levels of genetic diversity so that specific conservation actions can be undertaken for those populations. Private allelic richness will also be informative in distinguishing between species and between different populations within a species. Despite its utility, there is no doubt that private allelic richness is a function of sample size (Kalinowski, 2004). Thus, additional sampling may reveal that many of the alleles that are currently recognized as private are actually not private.

The microsatellite loci that were used in our study, combined with previously developed microsatellite libraries (Fitzsimmons et al., 2001; Miles et al., 2009a,b), can be used to help conserve and manage these 4 species of Neotropical crocodiles. Microsatellites that can be successfully amplified in cross-species reactions can aid managers in identification of purebred populations as well as hybrid and admixed individuals. Because illegal hunting for skin and meat is a major threat to all Neotropical crocodiles, these markers can also be used as forensic tools for law enforcement to facilitate stricter regulations against illegal hunting (Targarona et al., 1996; Cedeño-Vázquez et al., 2012; Ponce-Campos et al., 2012). This suite of microsatellites will also help reduce the time and resources needed to characterize the genetic fingerprint of any particular species, thus adding microsatellites to the list of conservation tools that can be used in the management of Neotropical and other crocodile species.

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Table 5. Allele frequencies of 10 microsatellite loci in populations of *Crocodylus acutus* and *C. moreletii*.

Locus	Allele	Pop. 1[a]	Pop. 2[b]	Pop. 3[c]	Pop. 4[d]
CpP2201					
	191	0.06	-	-	0.06
	211	0.53	0.42	-	0.28
	215	-	0.08	0.50	0.22
	227	0.41	0.50	-	0.33
	231	-	-	0.44	0.11
	235	-	-	0.06*	-
CpP2202					
	188	0.44	0.46	0.50	0.55
	196	0.09	0.04	-	-
	200	0.47	0.50	0.50	0.45
CpDi06					
	224	-	-	0.53	0.11
	226	0.02*	-	-	-
	228	0.03*	-	-	-
	230	0.56	0.54	-	0.06
	242	-	-	0.47*	-
	244	-	-	-	0.11*
	246	0.05	-	-	0.22
	248	0.34	0.46	-	0.11
	258	-	-	-	0.06*
	260	-	-	-	0.06*
	262	-	-	-	0.06*
	264	-	-	-	0.22*
CpP208					
	179	0.69	0.54	0.53	0.56
	191	0.31	0.46	0.47	0.44
CpP314					
	238	0.02*	-	-	-
	242	0.02*	-	-	-
	246	-	-	0.09*	-
	254	0.03	-	0.16	-
	258	0.03	-	0.63	-
	262	-	-	0.06	0.10
	266	0.11	0.04	0.03	0.10
	270	0.22	0.31	-	0.50
	274	0.06	0.27	0.03	0.10
	278	0.03*	-	-	-
	282	0.47	0.38	-	0.20
	286	0.02*	-	-	-
CpP4311					
	175	0.03*	-	-	-
	187	0.22	0.08	-	-
	195	-	-	0.78	0.22
	199	0.53	0.81	0.06	-
	203	0.03*	-	-	-
	211	0.02	0.04	0.13	0.78
	215	0.08*	-	-	-
	219	0.09	0.08	0.03	-
CpP815					
	206	-	-	0.06*	-
	210	-	-	0.06*	-
	214	-	-	0.06*	-
	218	0.03	-	0.22	0.10
	222	-	-	0.41	0.10
	226	0.06	-	0.13	0.15
	230	0.31	0.35	0.03	-
	234	-	-	-	0.10*
	238	0.17	0.08	0.03	0.40
	242	-	0.19*	-	-
	246	0.42	0.38	-	0.15

Continued on next page

Table 5. Continued.

Locus	Allele	Pop. 1[a]	Pop. 2[b]	Pop. 3[c]	Pop. 4[d]
CpP801					
	141	0.05	0.17	-	-
	145	-	-	0.06*	-
	149	-	-	0.47*	-
	153	0.06	-	0.09	-
	157	0.02	-	0.03	0.06
	161	0.06	0.21	0.06	-
	165	0.17	0.13	0.09	0.22
	169	0.03	-	0.09	0.06
	173	0.11	0.04	0.09	0.11
	177	0.28	0.29	-	0.50
	181	0.03*	-	-	-
	185	-	0.04	-	0.06
	189	0.05*	-	-	-
	193	-	0.04*	-	-
	197	0.14*	-	-	-
	201	-	0.08*	-	-
CpDi04					
	142	0.48	0.45	-	0.07
	144	0.06	0.10	0.97	0.07
	146	0.02*	-	-	-
	148	-	-	-	0.07*
	154	0.37	0.35	-	0.14
	156	0.02	0.10	0.03	0.57
	160	0.02*	-	-	-
	162	0.02*	-	-	-
	166	0.02	-	-	0.07
CpP1306					
	77	0.02*	-	-	-
	81	0.05	0.05	0.09	-
	85	0.33	0.25	-	-
	89	0.03	0.10	-	-
	93	0.19	0.40	-	0.06
	97	0.05	-	0.03	-
	101	0.06	-	0.09	-
	105	0.17	-	-	0.19
	109	0.03	0.15	0.50	0.19
	113	0.05	-	0.16	0.44
	117	-	0.05*	-	-
	121	-	-	0.03	0.13
	129	0.02*	-	-	-
	149	-	-	0.09	-
	153	0.02*	-	-	-

[a]*C. moreletii* - Belize (N = 32); [b]*C. moreletii* - Mexico (N = 13); [c]*C. acutus* - Panama (N = 16); [d]*C. acutus* - Mexico (N = 10). *Private allele for that particular locus in that particular population.

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