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Microsatellite DNA Markers Applied to Detection of Multiple Paternity in *Caiman latirostris* in Santa Fe, Argentina

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ABSTRACT Detecting multiple paternity in wild populations of the broad-snouted caiman (*Caiman latirostris*) has important implications for conservation efforts. We have applied microsatellite markers to examine genetic variation in *C. latirostris* and also have provided the first data concerning detection of multiple paternity in wild populations of this species. Blood samples from four nest-guarding *C. latirostris* females and their hatchlings were obtained from Santa Fe Province, Argentina. Amplified products were analyzed by electrophoresis on 10% polyacrylamide gels and visualized with silver staining. Four out of the eight markers tested reliably amplified and yielded useful data. Using polyacrylamide gels with silver staining provides high enough resolution to obtain individual genotypes. In order to assess the presence or absence of more than two parents in each nest, we used the single locus Minimum Method, and applied Cervus 3.0 and Gerud 2.0 software in parentage analyses. Our results indicate more than one father in at least two families. This behavior could be the consequence of high habitat variability in the area where our population was sampled. The ability to understand mating systems is important for maintaining viable populations of exploited taxa like *C. latirostris*. *J. Exp. Zool. 309A:637–642, 2008.*

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The broad-snouted caiman (*Caiman latirostris*) is a crocodilian that is indigenous to Argentina, Bolivia, Brazil, Paraguay and Uruguay. This species is a member of the family Alligatoridae and lives preferably in shallow aquatic environments and vegetated marshes within the Paraná river basin (Larriera, '92). Since 1990, wild populations of *C. latirostris* in Santa Fe province, Argentina have been managed by Proyecto Yacaré (Min. Prod./MUPCN), a program whose primary goal is the conservation of habitat through developing sustainable use of the broad-snouted caiman. In 1997, Argentinean populations of this species were changed from Appendix I to II of CITES. Populations of *C. latirostris* have since increased, and regulated trade has been allowed under a ranching system (Larriera, '98). Now, *C. latirostris* has become a commercially relevant species at both national and international levels.

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Recent population genetic studies of *C. latirostris* from Brazil have included microsatellite markers in their analyses (Verdade et al., 2002; Villela, 2004). Microsatellites, which are short tandem arrays of simple nuclear DNA sequences (Tautz and Schlötterer, '94), are often the molecular marker of choice because they are highly polymorphic and found abundantly throughout the genome (Bruford and Wayne, '93). Verdade et al. (2002) used four microsatellite markers, originally developed for *Alligator mississippiensis*, in genetic variation analyses of *C. latirostris* populations and found microgeographic variation. More recently, Villela (2004) recorded high values of genetic diversity and heterozygosity in *C. latirostris* using microsatellite markers specifically developed for this species (Zucoloto et al., 2002; Zucoloto, 2003). These broad-snouted caiman primers were also successfully applied to amplification of DNA samples of *Paleosuchus palpebrosus*, *C. crocodylus* and *C. yacare* (Zucoloto et al., 2006).

This study aims to utilize microsatellite markers in order to understand the underlying genetic diversity and mating structure of *C. latirostris* in Santa Fe, Argentina, by following similar work conducted on other crocodilian species. For example, microsatellite markers have been applied in past studies of genetic variability and population structure of *Crocodylus moreletii* (Dever and Densmore, 2001; Dever et al., 2002) and *A. mississippiensis* (Glenn et al., '98, 2002; Davis et al., 2001a, 2002). Other authors have applied microsatellites in parentage testing in *Cr. porosus* (Isberg et al., 2004) and in analyses of mating patterns in *A. mississippiensis* (Davis et al., 2001b). Davis et al. (2001b) also inferred multiple paternity, ''the occurrence of offspring within a single clutch being fathered by more than one male,'' in seven of 22 clutches of *A. mississippiensis*. Data on reproductive behavior of *C. latirostris* are limited because mating occurs in the water between groups of males and females (which are not easily distinguishable) and unambiguous observations are not often possible (Lang, '89). Thus, microsatellite markers may be useful in helping to understand mating patterns of *C. latirostris*. This work provides an alternative methodology for working with microsatellites that are specifically designed for genetic material of the broad-snouted caiman. Multiple paternity is a mating strategy that increases genetic variability; therefore, populations showing this should be better adapted to selective pressures than populations with single paternity (Sugg and Chesser, '94).

METHODS AND MATERIALS

Four nest-guarding *C. latirostris* females from Santa Fe Province, Argentina, were captured, measured and weighed. Blood samples of these females were obtained by puncture of internal jugular vein near the cervical vertebra (Tourn et al., '93) and diluted (1:10) in lysis buffer (Longmire et al., '88) for long-term storage at room temperature according to White and Densmore ('92). Eggs from the nests were collected for artificial incubation at 31.5°C, with 95% relative humidity, at Proyecto Yacaré facilities in Santa Fe City. Five days after hatching, between 10 and 15 hatchlings were randomly collected from each nest. One blood sample of 0.5 mL was obtained from each hatchling using the same method used for the adult females. DNA was isolated as previously described by Murray and Thompson ('80).

Eight microsatellite primers developed by Zucoloto et al. (2002) for *C. latirostr*is were tested: *Cla*μ 2, *Cla*μ 5, *Cla*μ 6, *Cla*μ 7, *Cla*μ 8, *Cla*μ 9, *Cla*μ 10, as well as *Ami*μ 20 developed for *A. mississippiensis* by Glenn et al. ('98) (Table 1). PCR conditions were performed according to Zucoloto et al. (2002) with modifications as follows: amplifications were carried out in a final volume of 15 μL with 2 × of specific buffer from PCR Optimizer Kit of Invitrogen (Carlsbad, CA) (except using *Ami*μ 20), 1.2 μL of each primer (0.1 nM/μL), 0.6 μL dNTP mix (200 μM), 0.06 μL *Taq* DNA polymerase (5 U/μL) from Promega (Madison, WI) and 50 ng of genomic DNA. All the buffers from the PCR optimizer kit (Invitrogen) were tested in the amplification of *Cla*μ primers. PCR reactions using primer *Ami*μ 20 were performed with PCR standard buffer from Promega (Table 1). A negative control containing all reagents except genomic DNA was included in each set of reactions.

PCR reactions were performed in a PTC-100 thermal cycler (MJ Research, Watertown, MA). Amplifications were performed using several annealing temperatures between 55 and 65°C and three different cycling programs (Table 2). Successful amplification was confirmed by separation on 2% agarose gels in TBE 0.5 × buffer (0.89 M Tris, 0.89 M boric acid and 0.11 M EDTA, pH 8.3). Gels were stained with ethidium bromide and analyzed under UV light. We assumed amplification was successful when we obtained bands with

TABLE 1. Tested primers: sequences and optimal buffers

Primer	5′–3′ sequence	Optimal buffer
*Cla*μ 2a	CCT TCA GGA CCC ACT TTC TT	A (7.5 mM $MgCl_2$, pH 8.5)
*Cla*μ 2b	CGA ATC CCT CTT CCC AAA CT	
*Cla*μ 5a	GCG TAG ACA GAT GCA TGG AA	F (10 mM $MgCl_2$, pH 9.0)
*Cla*μ 5b	CAG TCT GAA GCT AGG GCA AA	
*Cla*μ 6a	GAA ATA TGG GAC AGG GAG GA	J (10 mM $MgCl_2$, pH 9.5)
*Cla*μ 6b	GGT TGG CTG CAT GTG TAT GT	
*Cla*μ 7a	CGG GGT CTT GGT GTT GAC TA	F (10 mM $MgCl_2$, pH 9.0)
*Cla*μ 7b	CGG GAC CAG GAG CTG TAT AA	
*Cla*μ 8a	CAG CCA CTG AAG GAA TTG AC	F (10 mM $MgCl_2$, pH 9.0)
*Cla*μ 8b	CAC ATA CCT GAC CCA GCT TAT C	
*Cla*μ 9a	ACA GGG GAA AAG AAG AGC TG	A (7.5 mM $MgCl_2$, pH 8.5)
*Cla*μ 9b	AAA ATC CCC CAC TCT TAC CC	
*Cla*μ 10a	TGG TCT TCT CTT CGT GTC CT	A (7.5 mM $MgCl_2$, pH 8.5)
*Cla*μ 10b	ATG AGC CCC TCT ATG TTC CT	
*Ami*μ20a	TTT TTC TTC TTT CTC CAT TCT A	Standard (2.5 mM $MgCl_2$, pH 8.3)
*Ami*μ20b	GAT CCA GGA AGC TTA AAT ACA T	

TABLE 2. Best PCR conditions evaluated for each microsatellite

Microsatellites	Step 1	Step 2	Final extension
*Cla*μ 5, *Cla*μ 9 and *Cla*μ 10	15 cycles (1 min) 94°C (1 min) 64°C (1 min) 72°C	20 cycles (1 min) 94°C (1 min) 60°C (0.45 min) 72°C	(7 min) 72°C
*Cla*μ 2, *Cla*μ 6 and *Cla*μ 7	35 cycles (1 min) 94°C (1 min) 60°C (1 min) 72°C		(7 min) 72°C
*Cla*μ 8 and *Ami*μ 20	35 cycles (1 min) 94°C (1 min) 57°C (1 min) 72°C		(7 min) 72°C

estimated molecular weights within the range established by Zucoloto et al. (2006). Products were analyzed by electrophoresis on 10% polyacrylamide gels at 2,200 V and 75 W and stained with $AgNO_3$ solution (Bassam et al., '91). Molecular weights in base pairs (bp) were estimated using a 100 bp ladder. We also used CTT Allelic Ladder Mix, FFv Allelic Ladder Mix and STR III Allelic Ladder Mix (Gene Print Silver STR III System) from Promega, as other methods for estimating molecular weight.

Allele counts and genotype were used to test for the presence or absence of more than two parents in each nest (single locus Minimum Method). This method assigns multiple paternity within a clutch, assuming that all alleles not accounted for by the maternal genotype were contributed by ''fathers'' (Myers and Zamudio, 2004). We assumed multiple paternity when the maternal genotype could be reconstructed and three or more additional alleles were present in the clutch. Data analyses were carried out using Cervus 3.0 (Marshall et al., '98) and Gerud 2.0 (Jones, 2005). Cervus 3.0 is a program that examines genetic data from codominant genetic markers and performs parentage analyses. Parentage tests (paternity) were performed in two steps, first using all individuals together, and then again as family groups (one female with at least ten hatchlings). Gerud 2.0 is a program that reconstructs parental genotypes from half-sib progeny arrays with known or unknown parents. Using each family genotype group and allelic frequencies, we performed a determination of compatible mothers and then tested all possible paternal genotypes to see which minimum combination of fathers can best explain the data set. We calculated relative probabilities based on allele frequencies and Mendelian segregation.

RESULTS

All females and hatchlings were successfully genotyped using four out of the eight microsatellite markers: *Cla*μ 2, *Cla*μ 6, *Cla*μ 9 and *Cla*μ 10. The other four primers amplified nonspecific bands that complicated the analysis on gels, and therefore were not included in subsequent analyses. Amplified products from each primer set showed four alleles each, varying in size from 139 bp (*Cla*μ 9) to 220 bp (*Cla*μ 10). Detected alleles were as follows: Locus *Cla*μ 2: 204, 206, 210 and 214; Locus *Cla*μ 6: 159, 161, 163 and 165;

Locus *Cla*μ 9: 139, 164, 166 and 168; Locus *Cla*μ 10: 214, 216, 218 and 220.

Applying the Minimum Method we found multiple paternity in two of the four analyzed families (families 1 and 4). Using Cervus 3.0 for the first parentage analysis we considered only offspring and candidate mother genotypes (the female guarding the nest), and found that all families were successfully assigned to the candidate mother. Only six (12.3%) of the 49 offspring tested were excluded from the analysis because they were typed at fewer than four loci. There were three inconsistent genotypes among known mother–offspring pairs, all of which occurred in locus *Cla*μ 2 of family 2, which we attribute to misinterpretation of the obtained bands. The parentage analysis was initially carried out using one simulated father in each particular family; the results showed an average observed assignment rate of 86% at both 80 and 95% confidence levels. Then, using two simulated candidate fathers, the observed average assignment rate increased to 93%, at both 80 and 95% confidence levels (Table 3). In three of the four analyzed families (families 1, 2 and 4), the observed assignment rate was higher considering two candidate fathers at 95% confidence level. Utilizing Gerud 2.0 we found that in three of the four analyzed families the candidate mother was determinated to be the most likely mother for all offspring. We determined that in family 2 there appeared to be more than one compatible mother, which corresponded to the inconsistency found using Cervus 3.0. In one family the clutch had an assignment minimum of two fathers (family 1). In the families 3 and 4, the clutch had an assignment minimum of one father and yielded a total of two minimum-father solutions.

DISCUSSION

Microsatellites were applied for the first time in *C. latirostris* samples collected from Santa Fe, Argentina. Genotypes were successfully detected using the silver-stained polyacrylamide gel method. This shows that the common, but more expensive fluorophore-based primer labeling method with genotyping performed on a sequencer can be substituted by less costly assays. Most of the alleles obtained corresponded to the established range of molecular weights in *C. latirostris* found by Zucoloto et al. (2006).

According to paternity analyses, more than one father was detected in at least two *C. latirostris* nests. The Minimum Method does not account for multiple paternity by males with similar genotypes (Myers and Zamudio, 2004); therefore, using this method we found evidence of more than one father in two of four nests. Multiple paternity was detected in three nests at both confidence levels (80 and 95%) when applying parentage tests using Cervus 3.0. This is owing to a higher observed assignment rate when considering two candidate fathers as opposed to only one father. When using

TABLE 3. Observed and expected assignment rates in each family using Cervus 3.0

	Candidate fathers	1	2
	Level	Assignment rate observed (expected)	Assignment rate observed (expected)
Family 1	Strict	21% (64%)	36% (66%)
	Relaxed	71% (87%)	86% (87%)
	Unassigned	29% (13%)	14% (13%)
	Total	100% (100%)	100% (100%)
Family 2	Strict	50% (58%)	60% (61%)
	Relaxed	80% (86%)	90% (85%)
	Unassigned	20% (14%)	10% (14%)
	Total	100% (100%)	100% (100%)
Family 3	Strict	0% (32%)	0% (31%)
	Relaxed	67% (74%)	78% (77%)
	Unassigned	33% (26%)	22% (23%)
	Total	100% (100%)	100% (100%)
Family 4	Strict	30% (71%)	50% (71%)
	Relaxed	50% (92%)	80% (92%)
	Unassigned	50% (8%)	20% (8%)
	Total	100% (100%)	100% (100%)

Gerud 2.0 we found that one family had a minimum of two fathers and two other families showed a high probability of having only two fathers. The obtained results using these different programs were not perfectly consistent, probably because Gerud uses a strict minimum criterion for which it produces a very conservative result, whereas Cervus produces the most likely result (Jones and Ardren, 2003).

Although our sample size is smaller, we were able to detect a higher level of multiple paternity in *C. latirostris* than previously reported by Davis et al. (2001b) for the American alligator. This phenomenon could be the result of a greater variability in environmental conditions, including frequent flooding and droughts affecting the population in our study area. Changes in habitat and climate are known to increase variability (Nevo, 2001), and it may be that multiple paternity is correlated to an increase in environmental variability. In order to have greater confidence in the occurrence of multiple paternity, it would be prudent to sample all males (potentially candidate fathers) found within the vicinity of the nests and to try to establish which of these possible fathers had a higher genetic contribution to the clutch. Additionally, further investigations conducted on *C. latirostris* will include expanding the number of families and the number of loci used in these analyses to verify the high level of multiple paternity found in our study.

Loss of genetic diversity has been associated with an increase in vulnerability to population depletion and also a higher extinction risk (Lande and Shannon, '96; Frankham, 2005). This is applicable to other crocodilian species, because of population reductions owing to overexploitation in the 1950s and 1960s (Thorbjarnarson, '99), and the concomitant decreases in genetic variability that typically follow such reductions. The use of multiple paternity as a mating strategy contributes to greater genetic variability as a population is recovering, as we assume there is also an increase in effective population size (Sugg and Chesser, '94). Maintaining sufficient genetic variability within wild populations helps to increase the ability of populations to respond to selective pressures and adapt to changing environmental conditions (Frankham, 2005). It is clear that understanding mating systems is important in maintaining viable populations of exploited taxa like *C. latirostris*. This knowledge will in turn help to indicate the proportion of males and females needed for reintroduction into the wild in order to maximize effective population size. Further studies on mating systems employing both a genetic and behavioral framework are now needed in order to better understand the reproductive biology of *C. latirostris*.

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LITERATURE CITED

Bassam BJ, Caetano-Anolles G, Gresshoff PM. 1991. Fast and sensitive silver staining of DNA in polyacrylamide gels. Anal Biochem 196:80–88.

Bruford MW, Wayne RK. 1993. Microsatellites and their application to population genetic studies. Curr Opin Genet Dev 3:939–943.

Davis LM, Glenn TC, Elsey RM, Brisbin Jr IL, Rhodes WE, Dessauer HC, Sawyer RH. 2001a. Genetic structure of six populations of American alligators: a microsatellite analysis. In: Grigg G, Seebacher F, Franklin CE, editors. Crocodilian biology and evolution. Chipping Norton: Surrey Beatty & Sons. p 38–50.

Davis LM, Glenn TC, Elsey RM, Dessauer HC, Sawyer RH. 2001b. Multiple paternity and mating patterns in the American alligator, *Alligator mississippiensis*. Mol Ecol 10:1011–1024.

Davis LM, Glenn TC, Strickland DC, Guilette LJ, Elsey RM, Rhodes WE, Dessauer HC, Sawyer RH. 2002. Microsatellite DNA analyses support an east–west phylogeographic split of American alligator populations. J Exp Zool (Mol Dev Evol) 294:352–372.

Dever JA, Densmore LD. 2001. Microsatellites in Morelet's crocodile (*Crocodylus moreletii*) and their utility in addressing crocodilian population genetic questions. J Herpetol 35:541–544.

Dever JA, Strauss RE, Rainwater TR, McMurry ST, Densmore LD. 2002. Genetic diversity, population subdivision, and gene flow in Morelet's Crocodile (*Crocodylus moreletii*) from Belize, Central America. Copeia 2002:1078–1091.

Frankham R. 2005.Genetics and extinction. Biol Conserv 126:131–140.

Glenn TC, Dessauer HC, Braun MJ. 1998. Characterization of microsatellite DNA loci in American Alligators. Copeia 1998:591–601.

Glenn TC, Staton JL, Vu AT, Davis L, Alvarado Bremen JR, Rhodes WE, Brisbin LI, Sawyer RH. 2002. Low mitochondrial DNA variation among American alligators and a novel non-coding region in crocodilians. J Exp Zool (Mol Dev Evol) 294:312–324.

Isberg SR, Chen Y, Barker SG, Moran C. 2004. Analysis of microsatellites and parentage testing in saltwater crocodiles. J Hered 95:445–449.

Jones AG. 2005. GERUD2.0: a computer program for the reconstruction of parental genotypes from half-sib progeny

arrays with known or unknown parents. Mol Ecol Notes 5:708–711.

Jones AG, Ardren WR. 2003. Methods of parentage analysis in natural populations. Mol Ecol 12:2511–2523.

Lande R, Shannon S. 1996. The role of genetic variation in adaptation and population persistence in a changing environment. Evolution 50:434–437.

Lang JW. 1989. Social behavior. In: Ross CA, editor. Crocodiles and alligators. New York: Facts on File, Inc. p 102–117.

Larriera A. 1992. La conservación y el manejo de *Caiman latirostris* en la Argentina. In: Verdade LM, Lavorenti A, editors. Anais do II Workshop sobre Conservaçao e Manejo do Jacaré-de-Papo-Amarelo (*Caiman latirostris*). Universidade de Sao Paulo, Piracicaba: CIZBAS/ESALQ. p 8–17.

Larriera A. 1998. The *Caiman latirostris* ranching program in Santa Fe, Argentina: the first commercial rearing 1998. Crocodiles. Proceedings of the 14th Working Meeting of the Crocodile Specialist Group, IUCN—The World Conservation Union. Gland, Switzerland and Cambridge, UK. p 379–385.

Longmire JL, Lewis AK, Brown NC, Buckingham JM, Clark LM, Jones MD, Meincke LJ, Meyne J, Ratliff RL, Ray FA, Wagner RP, Moyzis RK. 1988. Isolation and molecular characterization of a highly polymorphic centromeric tandem repeat in the family Falconidae. Genomics 2: 14–24.

Marshall TC, Slate J, Kruuk L, Pemberton JM. 1998. Statistical confidence for likelihood-based paternity inference in natural populations. Mol Ecol 7:639–655.

Murray MG, Thompson WF. 1980. Rapid isolation of high molecular weight plant DNA. Nucleic Acid Res 8:4321–4325.

Myers EM, Zamudio KR. 2004. Multiple paternity in an aggregate breeding amphibian: the effect of reproductive skew on estimates of male reproductive success. Mol Ecol 13:1951–1963.

Nevo E. 2001. Evolution of genome–phenome diversity under environmental stress. Proc Natl Acad Sci 98:6233–6240.

Sugg DW, Chesser RK. 1994. Effective population size with multiple paternity. Genetics 137:1147–1155.

Tautz DC, Schlötterer C. 1994. Simple sequences. Curr Opin Genet Dev 4:832–837.

Thorbjarnarson J. 1999. Crocodile tears and skins: international trade, economic constraints and limits to the sustainable use of crocodilians. Cons Biol 13:465–470.

Tourn S, Imhof A, Costa AL, von Finck MC, Larriera A. 1993. Colecta de sangre y procesamiento de muestras en Caiman latirostris (Informe de avance). In: Larriera A, Imhof A, von Finck MC, Costa AL, Tourn SC, editors. Memorias del IV Workshop sobre Conservación y Manejo del yacaré overo (*Caiman latirostris*). Santa Fe: Fundación Banco Bica. p 25–30.

Verdade LM, Zucoloto RB, Coutinho LL. 2002. Microgeographic variation in *Caiman latirostris*. J Exp Zool (Mol Dev Evol) 294:387–396.

Villela PMS. 2004. Caracterização genética de populações de jacaré-de-papo-amarelo (*Caiman latirostris*), utilizando marcadores microssatélites. Unpubl. MS Thesis. Escola Superior de Agricultura ''Luiz de Queiroz'', Universidade de São Paulo, Piracicaba, São Paulo, Brazil.

White PS, Densmore III LD. 1992. Mitochondrial DNA isolation. In: Hoelzel AR, editor. Molecular genetic analysis of populations. A practical approach. Oxford: Oxford University Press. p 29–57.

Zucoloto RB. 2003. Desenvolvimento de seqüencias de DNA microssatélite para estudo de populações remanescentes de Jacaré-de-Papo Amarelo (*Caiman latirostris*), da região central do estado de São Paulo. Unpublished Ph.D. Thesis, CENA, Universidade de São Paulo, Piracicaba, São Paulo, Brazil.

Zucoloto RB, Verdade LM, Coutinho LL. 2002. Microsatellite DNA library for *Caiman latirostris*. J Exp Zool (Mol Dev Evol) 294:346–351.

Zucoloto RB, Villela PMS, Verdade LM, Coutinho LL. 2006. Cross-species microsatellite amplification in South American caimans (*Caiman* spp and *Paleosuchus palpebrosus*). Gen Mol Biol 29:75–78.