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Mating System of *Caiman yacare* (Reptilia: Alligatoridae) Described from Microsatellite Genotypes

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Abstract

The yacare caiman (*Caiman yacare*) is a reptile from South America and 1 of the 2 crocodilian species present in Argentina. The degradation of their natural habitat and strong hunting pressure led to a sharp numerical decline of wild populations. Nowadays, *C. yacare* is included in Appendix II of CITES, and ranching practices in some areas in Argentina are helping hatching success. In this context, it is important to better understand the population structure and mating system of the species. To do this, we amplified 10 microsatellite markers (SSRs) in 148 individuals of 13 *C. yacare* nests. All of the markers were polymorphic with 2–12 alleles per locus, with allelic sizes ranging between 154 and 400 bp and medium levels of polymorphism (H_o = 0.152–0.551 and H_e = 0.221–0.621). We were able to determine the maternal genotype in 9 out of 13 nests. In 6 of them we found more than 1 paternal genotype, with a maximum of 3 fathers for a single nest. This study is the first to provide evidence of multiple paternity behavior. These findings will be useful to improve management and conservation strategies for the species.

Subject area: Conservation genetics and biodiversity; Reproductive strategies and kinship analysis
Key words: *Caiman yacare*, CITES, multiple paternity, SSR, sustainable use

The family Alligatoridae is composed of 4 genera: *Alligator*, *Caiman*, *Melanosuchus*, and *Paleosuchus*, distributed exclusively in the Americas, except for the species *Alligator sinensis*, which is found in China (Ross 1998). In Argentina, 2 species of *Caiman* are present: *C. yacare* (yacare caiman) and *Caiman latirostris* (broad-snouted caiman). The geographic distribution of both species is quite similar but that of *C. latirostris* is extended further south. *Caiman yacare* is one of the most abundant crocodilian species in South America. The species can be found in the Amazon River basin of Bolivia and the Paraguay-Paraná River basin of Brazil, Paraguay, and Argentina (Godshalk 2008) (Figure 1). In Argentina, *C. yacare* has been reported in Chaco, Formosa, Corrientes, and Santa Fe provinces (Larriera and Imhof 2000). Although *C. yacare* hunting in Argentina is currently prohibited by the National Conservation of Fauna Law N° 22,421

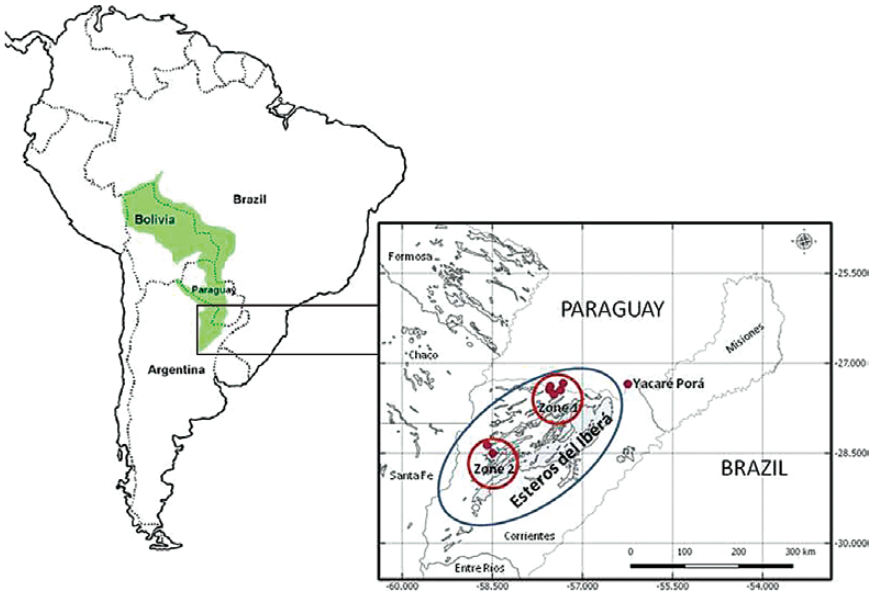

Figure 1. Geographical distribution for *Caiman yacare* (Godshalk 2006). Nests' collection per ranching (Zone 1 and Zone 2). "Esteros del Ibera" (the wetland). "Yacaré Porá" (breeding farm).

Decree 666/97 (Waller and Micucci 1995), this species was indiscriminately hunted for trade for more than 50 years, due to the high cost of its leather, and thus its were populations greatly reduced (Waller and Micucci 1993, 1995). Besides, the agricultural and urban expansion that has taken place in the last decades has caused habitat loss for many species, including *C. yacare* (Moreno and Parera 1997, 1998; Prado et al. 2000; Larriera et al. 2006; Larriera et al. 2008). Thus, the implementation of sustainable use programs of wild species could help to protect and prevent loss of natural areas (Larriera et al. 2006; Larriera et al. 2008). In 2005, a management program for the 2 *Caiman* species present in Argentina began in the province of Corrientes. The hatchery "Yacaré Porá" is a model of conservation of wild species (Larriera et al. 2008). Currently, *C. yacare* populations are evidencing signs of recovery and can be found in Appendix II of the Convention on International Trade in Endangered Species of Flora and Fauna (CITES) (Larriera et al. 2008).

Nowadays, microsatellites (SSRs) are some of the nuclear markers most used in population genetics, particularly in wild species, for the study of intra- and interspecific variation, lineage analysis and reproductive systems. Microsatellites have been used in both the genus *Alligator* (Glenn et al. 1998, Davis et al. 2000, 2002) and other Crocodilian species (Fitzsimmons et al. 2000, 2002; Davis et al. 2002), where they demonstrated high variability values. Microsatellites have also been used to elaborate genealogies and to study the mating system of crocodilians (Davis et al. 2001; Isberg et al. 2004a; Mc Vay et al. 2008). Evidence of multiple paternity has been shown in some species of crocodiles, including: *Alligator mississippiensis* (Davis et al. 2001; Lance et al. 2009), *A. sinensis* (Hu and Wu 2010), *Crocodylus moreletii* (McVay et al. 2008), *Melanosuchus niger* (Muniz et al. 2011), *C. latirostris* (Amavet et al. 2008, 2012), *Crocodylus porosus* (Lewis et al. 2013), *Caiman crocodilus* (Oliveira et al. 2014), *Crocodylus acutus* (Budd et al. 2015) and *Crocodylus intermedius* (Rossi Lafferriere et al. 2016).

In his PhD thesis, Godshalk (2006) designed specific microsatellite primers for *C. yacare*, but no population genetic studies have yet been published. Other authors have designed specific primers for closely related species such as *C. latirostris* (Zucoloto et al. 2002; Amavet et al. 2015) and *C. crocodilus* (Oliveira et al. 2010), and successfully amplified them in *C. yacare*.

Monitoring reproductive interactions in animals in their natural habitat is sometimes difficult due to the different types of mating behaviors. Paternity analysis allows learning about the mating behavior of species because it provides an indirect approach to study their breeding biology. The use of molecular markers is a good approach to assign feasible parents in the offspring (Fleischer 1996; Fitzsimmons 1998).

To our knowledge, there are no studies about the reproductive biology or breeding system of *C. yacare* nor about general genetic diversity parameters of this species in Argentina. These data would be very relevant and useful to develop management and conservation programs. Based on the fact that these reptiles adopt multiple paternity as a strategy in their mating system, the aim of this study was to assess multiple paternity in *C. yacare* by the use of microsatellite genotyping and genetic analysis.

Materials and Methods

Sample Collection and DNA Extraction

Samples were collected from "Yacaré Porá," a hatchery of *C. yacare* located in Corrientes, Argentina. This farm is located in the northeast region, close to "Esteros del Iberá," one of the largest wetlands in the world, where there are 4000 species of plants and animals, that is, 10% of all species recorded in the continental aquatic environments in the world (Neiff and Neiff 2013) (Figure 1). "Yacaré Porá" uses the ranching methodology, which is the technique recommended by the Crocodile Specialist Group (CSG) of the Species Survival Commission (SSC) and the International Union for Conservation of Nature (IUCN), due to its good results on the recovery of crocodilian populations and benefits for local people. Under the ranching system, the eggs are collected from the wild (thus, there is no information about their potential parents) and then incubated under controlled temperature (30–32 °C) and humidity (95–98%) (Larriera et al. 2008).

A total of 148 blood samples from animals hatched from eggs from 5 nests collected in 2012 and another eight nests collected in 2013 within 2 areas separated by approximately 180 km (Zones 1 and 2; Figure 1) were collected for our study. There were no differences between the structures that made up the atmosphere between zones. Both areas had similar climatic conditions and are within the Argentine wetland "Esteros del Iberá." Zone 1 has a more pristine environment, whereas Zone 2 is close to some urban settlements. Nests N-345, N-310, N-302, N-293, N-211, N-91, N-498, N-494, N-508, and N-509 were collected in Zone 1, whereas nests N-450, N-459, and N-26 were collected in Zone 2.

The samples were taken from hatchlings within 24 h after hatching; approximately 30–40% of the nest was used after artificial incubation, so we had no information about their potential parents. At 9 months, some of the hatchlings are released into the wild (the area where each nest was extracted from) whereas some others are used for commercial breeding.

Blood samples (0.1 mL) were obtained by puncture of the internal jugular vein near the cervical vertebra (Tourn et al. 1993) (n = 7–12 individuals/nest). Blood was then diluted (1:10) in lysis buffer (Longmire et al. 1988) for long-term storage at room temperature, according to White and Densmore (1992). DNA was isolated based on the protocols of Murray and Thompson (1980) modified based on Amavet et al. (2012), obtaining a final concentration of 50 ng/µL.

Microsatellite Genotyping

For genotyping, 10 microsatellite markers were evaluated: 4 primer pairs corresponding to markers developed by Zucoloto et al. (2002) for *C. latirostris* (*Cla*µ 2, *Cla*µ 5, *Cla*µ 6, and *Cla*µ 10); 4 primer pairs developed by Godshalk (2006) for *C. yacare* (R-8, N-10, I-14, and O-22); and 2 primer pairs developed by Amavet et al. (2015) for *C. latirostris*: (*Cl*-236 and *Cl*-58) (Table 1). Genotyping reactions were carried out in a Bioer Life Express® thermal cycler with gradient of 96 wells. Each polymerase chain reaction (PCR) contained: 2 µL of PCR 10× buffer PB-L® (100 mM Tris–HCl, pH 8.5, 500 mM KCl), 0.6–1 µL of $MgCl_2$ 50 Mm PB-L®, dNTPs 20 mM PB-L®, 1 µL each of the fluorescent label forward primer and unlabeled reverse primer, 0.5 U Taq DNA polymerase PB-L®, and 1 µL of DNA template (50 ng/µL). Finally, 14.08–15.06 µL of milliQ water was added to a final reaction mix volume of 20 µL. A negative control was included in each PCR reaction.

The amplifications were performed according to the following cycling program: initial denaturation for 5 min at 94 °C and then 30 cycles with denaturation at 94 °C for 60 s, annealing at optimum temperature for each marker (Table 1) and extension at 72 °C for 75 s. A final extension at 72 °C for 10 min completed each reaction. PCR products were analyzed on 2% agarose gels stained with Gel Green® and subsequently visualized on a Dark Reader® transilluminator. To genotype the PCR samples, we used 2 techniques: 1) electrophoresis on 10% polyacrylamide gels and 2) genotyping service.

Genotyping by Electrophoresis

PCR products of the 4 primers developed by Zucoloto et al. (2002) were analyzed by electrophoresis on 10% polyacrylamide gels of 33 cm × 39 cm, at 2200 V and 75 W in 0.5X TBE buffer (Tris, Boric acid, EDTA—Promega®), stained with silver nitrate solution (Bassam et al. 1991). Every 20 samples, a ladder was placed 10 bp (Invitrogen®). Stained gels were photographed with a digital camera (Olympus C5000) using the macro-mode. For allele size (in bp), CorelDRAW Graphic Suite X3 Software was used, with reference to the size of the fragments of the ladder.

Automatic Genotyping

PCR products using primers developed by Godshalk (2006) and Amavet et al. (2015) were genotyped in Macrogen, Inc. (Korea). Fragment lengths were assigned to allelic classes with Peak Scanner® 1.0 Software (Applied Biosystems) and then scored for length using GS500LIZ_3730 as the internal lane-size standard.

Table 1. Locus microsatellite, primers, repeat motif, size range (bp), allele number (*NA*), observed heterozygosity (*Ho*), expected heterozygosity (*He*), label or genotyping by electrophoresis (GE), (AT) annealing temperature, and source for the 10 microsatellite markers

Locus	Primer sequence (5′–3′)	Repeat motif	Size range (bp)	N_A	H_o	H_e	Label	AT (°C)	Source
*Cla*µ 10	F-TGGTCTTCTCTTCGTGTCCT R- ATGAGCCCCTCTATGTTCCT	$(CA)_{12}$, $(CT)_{19}$	168–174	4	0.359	0.221	GE	60	Zucoloto et al. (2002)
*Cla*µ 6	F-GAAATATGGGACAGGGAGGA R-GGTTGGCTGCATGTGTATGT	$(AC)_{17}$	170–200	7	0.152	0.243	GE	61	Zucoloto et al. (2002)
*Cla*µ 5	F-GCGTAGACAGATGCATGGAA R-CAGTCTGAAGCTAGGGCAAA	$(AC)_{16}$, $(AC)_{22}$	154–212	9	0.205	0.320	GE	65	Zucoloto et al. (2002)
*Cla*µ 2	F-CCTTCAGGACCCACTTTCTT R-CGAATCCCTCTTCCCAAACT	$(GA)_{16}$, $(GT)_{10}$	200–270	10	0.409	0.313	GE	66	Zucoloto et al. (2002)
R-8	F-GCCCAAGTTGAAGGTGTGTT R-AAGGGCAGAGTCCAGTTTCA	$(CA)_{19}$	218–242	10	0.250	0.479	FAM	62	Godshalk (2006)
N-10	F-TGCTGACCATTTTACTTCTTTGA R-CTTCCCCAGCAACCTGAATA	$(GT)_{27}$	280–320	12	0.242	0.568	FAM	62	Godshalk (2006)
I-14	F-CCCTCATCCCTCTTCAATCA R-GGCCAAACCAAAGTAAAGCA	$(GT)_{24}$	228–246	8	0.551	0.621	HEX	60.6	Godshalk (2006)
O-22	F-TCACCTTGAGTGAGCTGCAT R-GCCTGAATTTGGCTTGACAT	$(CA)_{26}$	174–192	6	0.451	0.504	HEX	57.8	Godshalk (2006)
Cl-236	F-TGAACCAGATGCCAGTGGAC R-TCCAACTGATCGCTGTCTCTG	$(GA)_6$	394–400	2	0.486	0.358	FAM	58	Amavet et al. (2015)
Cl-58	F-CTCTCGGCAGAAGCTACTGG R-AATGAATGCGCGTGTCTGTG	$(AC)_6$	380–388	4	0.283	0.379	HEX	60	Amavet et al. (2015)

Genetic Analysis

We constructed the compound genotypes for every individual. Relative probabilities based on allelic frequencies and Mendelian segregation were calculated by Micro-Checker v2.2.3 (Van Oosterhout et al. 2004). This software analyzes possible stuttering in the band size, amplification of the predominant allele (dropout), null alleles, and misprints.

To analyze genetic variability, we used the software GENALEX v6.4 (Peakall and Smouse 2006). In addition, we calculated the probability of excluding multiple paternity for each individual locus and the combined probability of all 10 loci.

The most likely maternal genotype was calculated using GERUD 2.0 (Jones 2005). Once we had the genotype of both the mother and the offspring, we used the option of known maternal genotype (GERUD 2.0) to calculate the parents of those individuals.

Allele counts and genotypes were used to test for the presence or absence of more than 2 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 paternities when the maternal genotype could be reconstructed and 3 or more additional alleles were present in the clutch (Amavet et al. 2008).

For paternity analysis, we used GERUD (Jones 2005). This software predicts compatible mothers, using genotypes and allelic frequency of each individual of each nest. The allelic frequencies of each genotyped nest were used to determine the compatible mothers with higher probability. Then, the different paternal genotypes were calculated to search the minimum parental combination, which would better explain the dataset. As the program does not accept missing data, the offspring that could not be genotyped at all loci were excluded. The program also makes no concessions for genotyping errors or mutations, but detects incompatibilities between parents and known offspring, indicating the rate of genotyping errors in the dataset, thereby allowing these data to be removed from the analyses.

Results

A total of 148 individuals from 13 nested were genotyped using 10 microsatellites. These markers were polymorphic with a range of 2–12 alleles per locus, and allelic sizes between 154 and 400 bp. Observed heterozygosity (H_o) values ranged from 0.150 to 0.551 and expected heterozygosity (H_e) values ranged from 0.221 to 0.621 (Table 1). These 10 loci were in Hardy–Weinberg equilibrium ($P < 0.005$).

When 7 loci were combined, the probability of paternity exclusion was 0.988, and the probability of genetic identity reached 0.002 (Table 2). The probability of paternity exclusion per locus ranged from 0.342 (*Cla*μ 6) to 0.683 (*Cl*-236), whereas the probability of genetic identity per locus ranged from 0.149 (*Cl*-236) to 0.678 (*Cla*μ 6).

The loci R-8, N-10, and I-14 showed signs of null alleles. These null alleles may be due to slippage during PCR amplification, which can produce additional stutter products that differ from the original template by multiples of the repeat unit length (Shinde et al. 2003). Such stutters are often common in dinucleotide loci, making it difficult to discriminate between homozygotes and heterozygotes. Finally, where mutations occurred at primer sites, certain alleles could not be amplified (null alleles), resulting in false homozygotes (Shaw et al. 1999).

In 4 nests, it was not possible to reconstruct the maternal genotype and were thus excluded from parental analysis. The inability to rebuild the maternal genotype could be due to the mixing of eggs during collection or to the possibility of the presence of eggs from different females in the nest, in agreement with that observed by Larriera (2002) in *C. latirostris*.

Using 7 of the 10 microsatellites, it was possible to determine the maternal genotype of 9 of the nests. These 7 microsatellites were considered enough to determine multiple paternity strategies in *C. yacare* (P2Max = 0.988), even when the parental genotypes were unknown. The GERUD analysis allowed assigning one father for each of 3 nests and at least 2 fathers for the other 6 nests. Nests 211, 293, 302, 345, 450, and 459 showed evidence of more than one father (2 or 3) per nest (Table 3). On the other hand, nests 494, 508, and 310 showed one father for all the offspring.

Discussion

This study provides the first approach to parentage and paternity of *C. yacare* in Argentina. Since microsatellite markers often provide information about the conservation status of the species, we can assume that the genetic conservation status of *C. yacare* is similar to that of other crocodilians. In this work, heterozygosity values were low, but these values should not be considered as population values because the samples were from 13 nests, which in turn were related

Table 2. Locus, number of alleles in all nests (*NA*), probability of exclusion, and probability of identity for each locus and for combinations of the 7 loci using GENALEX v6.4 (Peakall and Smouse 2006)

Locus	N_A	Probabilities of each locus		Locus combinations	Probabilities of locus combinations	
		PISibs 1	PX2		PISibs 2	P2Max
*Cla*μ 10	4	0.347	0.669	*Cla*μ 10	0.347	0.657
*Cla*μ 6	7	0.342	0.678	*Cla*μ 10 + *Cla*μ 6	0.119	0.871
*Cla*μ 5	9	0.403	0.531	*Cla*μ 10 + *Cla*μ 6 + *Cla*μ 5	0.048	0.941
*Cla*μ 2	10	0.522	0.312	*Cla*μ 10 + *Cla*μ 6 + *Cla*μ 5 + *Cla*μ 2	0.025	0.960
O-22	6	0.388	0.551	*Cla*μ 10 + *Cla*μ 6 + *Cla*μ 5 + *Cla*μ 2 +O-22	0.009	0.979
Cl-236	2	0.683	0.149	*Cla*μ 10 + *Cla*μ 6 + *Cla*μ 5 + *Cla*μ 2 +O-22 + *Cl*-236	0.006	0.982
Cl-58	4	0.583	0.254	*Cla*μ 10 + *Cla*μ 6 + *Cla*μ 5 + *Cla*μ 2 +O-22 + *Cl*-236 + *Cl*-58	0.003	0.988

PISibs 1, probability of identity for each locus; PX2, probability of exclusion for each locus; PISibs 2, probability of sibling identity for locus combinations; P2Max, probability of exclusion for locus combinations.

individuals, which would be producing some sort of inbreeding. For this reason, the values of H_e and H_o would be useful in future genetic studies in populations where the sample size can be expanded so as to cover the entire area of distribution. Because of the low genetic diversity levels obtained in this study, we consider that it would be especially important to monitor this species all over the distribution area.

In studies where a female lays the eggs in a nest, it is assumed that all the corresponding hatchlings are at least half siblings. Our results in 9 *C. yacare* nests showed a high polyandry rate (approximately 70%). These multiple paternity values are higher than those found in other crocodile species, such as *A. mississippiensis* (32%—Davis et al. 2001 and 52%—Lance et al. 2009), *C. moreletii* (50%—McVay et al. 2008), *C. latirostris* (50% and 24%—Amavet et al. 2008, 2012), *A. sinensis* (30%—Hu and Wu 2010), and *M. niger* (50%—Muniz et al. 2011). However, the highest frequency of multiple paternity reported is that for *C. crocodiles* (95%) (Oliveira et al. 2014).

Crocodilians have a mating system with clear male social domain, where some males contribute to the gene pool, a mechanism that may tend to decrease the genetic diversity of the population (Rowe and Hutchings 2003). Overexploitation can alter the mate choice and the dynamics of courtship (Allendorf and Hard 2009). In this species, the overexploitation that has occurred in recent decades has led to a decrease in the number of dominant males, and thus, due to this absence of dominant males, females could adopt a different behavior and randomly choose several males to mate. Moreover, there is evidence regarding the social domain in crocodiles, where the alpha male, a larger and stronger male, is the one that matches more effectively with females (Garrick and Lang 1977). It is for this reason that if these alpha males are removed from nature due to hunting or habitat fragmentation, their absence would have consequences on the genetic structure and social organization of the natural population (Lewis et al. 2013). The expansion of the agricultural frontier and the increase in habitat fragmentation negatively influence the structure of the population of reptiles (Gardner and Oberdörster 2005).

Multiple paternity in reptiles occurs when there are certain rates of female–male encounters (Uller and Olsson 2008). For instance, in turtles, when the mating group is dense, multi-paternity levels reach 90%, compared to 30% at low-density sites (Jensen et al. 2006). On the coast of Louisiana (USA), crocodiles reproduce at high densities; in this case, it is possible to find many individuals (Elsey et al. 2008).

In species where the population decreases due to poaching, habitat loss or an improper management of commercial programs, some mating behaviors may accelerate the rates to recover their populations, and multiple paternity seems to be a good alternative (Klemme et al. 2008). In *C. yacare*, the successful recovery of natural populations could be due to this mating strategy.

Gist et al. (2008) summarizes 2 events of polygamy in crocodiles: 1) several copulations (no storage of semen in the reproductive tract) between one reproductive period and the next, and 2) single and multiple copulations with sperm storage between reproductive periods. Although both events have been confirmed in *A. mississippiensis*, it is not possible to generalize this for all species. Alligators mate only once a season; therefore, it may not be necessary to store sperm for the subsequent reproductive season (Davis et al. 2001). *C. yacare* shows a similar reproductive behavior.

In the present work, considering the study area and density as determinant factors in mating systems, a polygamous behavior may be an important strategy to limit the inbreeding caused by hunting and habitat fragmentation. The habitat fragmentation, the expansion of the agricultural frontier, and the consequent decrease in their habitat could be causing agglomeration of individuals in small habitats, and this might be causing the high rate of multiple paternity found in this study.

Table 3. Parental genotypes using GERUD for each nest using 7 microsatellites: alleged mother, possible fathers, and the number of hatchlings assigned to each (#progeny)

Nest		*Clαμ* 10	*Clαμ* 6	*Clαμ* 5	*Clαμ* 2	O-22	*Cl*-236	*Cl*-58	#Progeny
Nest 211:	Mother	210/210	160/160	200/200	170/168	178/188	394/400	382/388	
(*n* = 12 ind)	Father1	214/214	160/160	200/200	170/168	178/188	400/400	382/384	9
	Father2	214/214	160/164	200/200	170/170	178/186	400/400	388/388	3
Nest 302:	Mother	200/250	160/160	194/200	170/170	174/178	394/400	382/382	
(*n* = 9 ind)	Father1	200/250	160/160	194/200	170/170	188/174	400/400	382/382	8
	Father2	250/250	160/160	194/194	170/170	178/178	394/394	382/382	1
Nest 345:	Mother	200/210	170/162	200/200	172/172	192/178	400/394	382/388	
(*n* = 12 ind)	Father1	200/200	170/162	200/200	172/172	186/178	400/400	382/382	7
	Father2	200/200	170/162	200/200	172/172	178/178	400/400	384/388	5
Nest 450:	Mother	210/260	170/170	170/176	170/172	178/178	400/400	382/388	
(*n* = 12 ind)	Father1	210/210	170/170	170/170	172/172	192/192	394/400	384/388	4
	Father2	210/210	170/170	170/176	172/172	178/188	394/400	384/382	8
Nest 459:	Mother	270/270	160/160	194/194	172/172	174/178	394/400	382/382	
(*n* = 12 ind)	Father1	270/270	160/154	194/194	172/170	174/174	400/400	388/388	2
	Father2	270/270	160/154	194/200	172/170	174/178	400/400	382/384	10
Nest 293:	Mother	250/200	156/204	200/200	172/172	192/188	394/400	388/382	
(*n* = 12 ind)	Father1	250/250	156/212	200/200	172/172	192/188	400/400	382/384	7
	Father2	250/250	212/212	200/200	172/172	176/174	400/400	382/382	2
	Father3	250/200	156/156	200/200	172/172	192/176	400/400	388/384	3
Nest 494:	Mother	210/250	154/160	198/198	172/172	174/178	394/400	382/382	
(*n* = 12 ind)	Father1	210/250	154/160	198/198	170/172	174/174	400/400	384/382	12
Nest 508:	Mother	210/220	160/168	194/200	172/172	178/192	394/400	384/384	
(*n* = 12 ind)	Father1	220/210	168/160	194/200	172/172	188/178	400/400	384/382	12
Nest 310:	Mother	244/244	154/164	200/194	170/168	174/188	400/394	382/380	
(*n* = 12 ind)	Father1	252/244	154/164	200/194	170/168	174/188	400/400	382/380	12

Multiple paternity as mating strategy must be considered a good indicator and an important factor in management programs for sustainable use and conservation of the species in the region. This behavior increases the genetic variability of the species, increasing the gene pool, and giving a better fitness against various environmental and human pressures (Young et al. 1996).

Today, it is common to see individuals of both species of caimans in east-central and northeastern Argentina (Prado et al. 2012). The successful recovery of populations of *C. caiman* is due in part to the laws controlling illegal trade but mainly to repopulation programs under the ranching system (Larriera et al. 2008).

As a result of the illegal indiscriminate hunting, Argentine caimans were decimated, producing a "genetic bottleneck," where caimans remaining after this event presented a lower genetic variability. The genetic variability can also be reduced by habitat fragmentation due to the gene flow between nearby populations. In other words, the loss of variability may reduce the ability of animals to deal with various environmental or anthropogenic situations. This report presents the first contribution regarding the mating system of *C. yacare* in Argentina, providing evidence of its reproductive biology, which may be useful for management programs and conservation plans. Multiple paternity strategies could increase the variability of caiman populations. Acquiring an increase in the gene pool would benefit their populations because they are apparently healthier in terms of fitness and would provide them with better tools to face adversity.

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Data Availability

We have deposited the primary data underlying these analyses as follows:

Sampling locations and microsatellite genotypes: Dryad (doi:10.5061/dryad.4s5r7).

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