Macrogeographic Genetic Variation in Broad-Snouted Caiman (*Caiman latirostris*)

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ABSTRACT   Broad-snouted caiman's (*Caiman latirostris*) geographic distribution comprises one of the widest latitudinal ranges among all crocodilians. In this study we analyzed the relationship between geographic distance (along the species latitudinal range) and genetic differentiation using DNA microsatellite loci developed for *C. latirostris* and *Alligator mississippiensis*. The results suggest that there is a consistent relationship between geographic distance and genetic differentiation; however, other biogeographical factors seem to be relevant. The Atlantic Chain (*Serra do Mar*) seems to be an effective geographic barrier, as well as the relatively narrow (≤ 1.5 km) sea channel between Cardoso Island and the continent. In addition, coastal populations seem to have been well connected in recent geological time (Pleistocene 16,000 years ago) all along the eastern Brazilian coast. Further studies should focus on the São Francisco River drainage, which is still poorly known for this species. *J. Exp. Zool. 309A, 2008.*   © *2008 Wiley-Liss, Inc.*

Broad-snouted caiman's (*Caiman latirostris*) geographic distribution comprises one of the widest latitudinal ranges among all crocodilians (Verdade and Piña, 2006). This can be dramatic for a large heterotherm (Pough et al., '98), as its growth rate and age at sexual maturity (Verdade and Sarkis-Gonçalves, '98; Verdade et al., 2003; Larriera et al., 2006) can vary two- or three-fold among populations from the lowest to the highest latitude.

Although crocodilians can move considerable distances through terra firma (Campos et al., 2006), watercourses are their primary dispersal pathways. Thus, on a larger scale, hydrographic basins usually determine distribution patterns of crocodilians (Sill, '68).

The broad-snouted caiman current distribution covers two major South American river basins, Paraná and São Francisco, as well as a number of small coastal drainages (Verdade and Piña, 2006). Paraná River runs southward, whereas São Francisco River runs northward and the small coastal rivers run mostly eastward. These geographic patterns can possibly affect the genetic flux among populations from different river drainages by affecting individual dispersal (Caughley and Sinclair, '94). In addition, either by recent anthropogenic pressure or due to historical events, there may be some genetic isolation among populations even on a microgeographic scale (Verdade et al., 2002). Therefore, genetic variation may be related to geographic distance—even on a small scale for this species. This hypothesis is tested in this study.

MATERIAL AND METHODS

Species definition

The broad-snouted caiman is a medium-sized crocodilian reaching a maximum total length of 2–3.5 m, proportionately broader than in other crocodilians (Verdade and Piña, 2006). The species is predominantly palustrine (Verdade, '98) and can be frequently found in artificial reservoirs on cattle ranches (Scott et al., '90).

Animal handling and blood collection

Field studies were carried out from October 1995 to December 2007. Capture techniques consisted of approaching animals by boat at night with a spotlight; juveniles (<1.0 m total length) were captured by hand, as described by Walsh ('87), and adults noosed, as described by Hutton et al. ('87). Captive animals were processed during daylight hours. Animals were immobilized using physical techniques without the use of tranquilizers (Verdade, '97; Huchzemeyer, 2003).

Blood was collected by puncturing the dorsal branch of the superior cava vein, which runs along the interior of the vertebral column of large reptiles (Olson et al., '75). Collected blood sample was stored in lysis buffer (Hoezel, '92): 100 mM Tris–HCl, pH 8.0; 100 mM EDTA, pH 8.0; 10 mM NaCl; 0.5% SDS (p v^{-1}).

Study sites and sampling effort

A total of 142 individuals were captured from 10 sites (Fig. 1, Table 1). Sampling locations were chosen to encompass the greatest area of geographic distribution for the broad-snouted caiman as described in Verdade and Piña (2006). Study sites ranged from the northernmost (Natal, in the state of Rio Grande do Norte) to the southernmost (Taim, in the state of Rio Grande do Sul) limits of the broad-snouted caiman geographic distribution (Verdade and Piña, 2006). The westernmost limit for the species in Brazil was also included (Bonito, in the state of Mato Grosso do Sul) as well as an insular population (Cardoso Island, off the Atlantic coast of the state of São Paulo). Blood samples collected in previous studies by Verdade ('97, 2001a), Verdade et al. (2002), Zucoloto (2003) and Villela (2004) were also included in this study.

Microsatellite analyses

Blood samples were digested with proteinase K to a final concentration of 0.5 mg mL^{-1}, proteins precipitated with 1.2 M NaCl and total DNA precipitated with ethanol (Hoezel, '92; Olerup and Zetterquist, '92). Eleven primer pairs were utilized, four (Amiμ8, Amiμ11, Amiμ13 and Amiμ20) developed by Glenn et al. ('98) for *Alligator mississippiensis* and seven (Claμ2, Claμ5, Claμ6, Claμ7, Claμ8, Claμ9 and Claμ10) developed by Zucoloto et al. (2002) for *C. latirostris*.

Fig. 1. Field sites. 1: Natal, Rio Grande do Norte (RN); 2: João Pessoa, Paraíba (PB); 3: Lagoa Vermelha, Alagoas (AL); 4: São Pedro Pantanal, São Paulo (PaT); 5: Charqueada, São Paulo (CH); 6: Porto de Areia, São Paulo (PoA); 7: Duraflora, São Paulo (DuF); 8: lha do Cardoso, São Paulo (IC); 9: Taim, Rio Grande do Sul (RS); and 10: Bonito, Mato Grosso do Sul (MS). Paraguay, Uruguay and Paraná Basins are part of the La Plata River Basin.

TABLE 1. Study sites and sampling effort

Site	Coordinates	n	Habitat type	No. of alleles	Source
Rio Grande do Norte	5°43′S, 35°12′W	5	Wetland	43	Villela (2004)
Paraíba	7°06′S, 34°52′W	12	Wetland	49	Villela (2004)
Alagoas	10°04′S, 36°21′W	25	Lake	74	Verdade (2001a)
Bonito	21°07′S, 56°30′W	10	Artificial reservoirs in cattle ranches	65	This study
Charqueada	22°30′S, 47°48′W	12	Artificial reservoirs in a cattle ranch	56	Verdade ('97) Verdade et al. (2002) Zucoloto et al. (2002)
Sao Pedro Pantanal	22°35′S, 47°51′W	20	Wetland	58	Verdade ('97) Verdade et al. (2002) Zucoloto et al. (2002)
Duraflora	22°26′S, 48°52′W	23	Artificial lakes in eucalyptus plantations	55	Verdade ('97) Verdade et al. (2002) Zucoloto et al. (2002)
Porto de Areia	22°39′S, 47°58′W	9	Lake	40	
Cardoso Island	25°04′S, 47°55′W	9	Creek	47	Villela (2004)
Rio Grande do Sul	32°32′S, 52°23′W	17	Wetland	46	

Amplification conditions were: polymerase chain reaction buffer (20 mM Tris–HCl, pH 8.4; 50 mM KCl), 1.5 mM $MgCl_2$, 0.2 mM each dNTP; 0.4 mM of each primer pair, 0.02 U mL^{-1} Taq DNA polymerase and 100 ng of DNA in a final volume of 25 μL. Amplification was as follows: (1) 94°C for 3 min, (2) 94°C for 45 sec, (3) primer pair annealing temperature for 1 min (Table 2), (4) 73°C for 1 min and 15 sec, (5) repeat steps (2), (3) and (4) n cycles according to Table 2, (6) 4°C indefinitely. Test phase amplifications were electrophoresed in 3% agarose gels, stained with ethidium bromide and visualized in a UV transilluminator. Sense primers were fluorescence-marked and amplification products were analyzed on a DNA MegaBace1000 sequencer (Molecular Dynamics, Sunnyvale, USA).

Genepop version 3.1d (Raymond and Rousset, '95) was used to determine allele frequency as well as the number of observed and expected heterozygotes according to Hardy–Weinberg equilibrium (HWE). Wright F statistics (F_{IS}, F_{ST}) were estimated using genetic data analysis (Lewis and Zaykin, 2007). According to Wright ('31), index ranges from 0 to 0.05, 0.05 to 0.15, 0.15 to 0.25 and >0.25, respectively, indicate low, moderate, high and very high genetic differentiation among populations.

The mutational processes in microsatellite loci differ from pattern assumed for infinite allele models as they present lower mutational rates. For this reason, we estimated R_{ST}, especially developed for microsatellites (Slatkin, '95) and considered such parameters as variance in allele size and relatively high mutation rates.

TABLE 2. Primers, amplification conditions (T = optimum annealing temperature (°C); C = number of PCR cycles) and number of alleles segregated for each primer

Primers	Sequencia 5′–3′	T	C	No. of alleles
*Ami*μ8a	CCTGGCCTAGATGTAACCTTC	55	30	3
*Ami*μ8b	AGGAGGAGTGTGTTATTTCTG			
*Ami*μ11a	AAGAGATGTGGGTGCTGCTG	64	35	10
*Ami*μ11b	TCTCTGGGTCCTGGTAAAGTGT			
*Ami*μ13a	CCATCCCCACCATGCCAAAGTC	64	35	17
*Ami*μ13b	GTCCTGCTGCTGCCTGTCACT			
*Ami*μ20a	TTTTTCTTCTTTCTCCATTCTA	58	30	18
*Ami*μ20b	GATCCAGGAAGCTTAAATACAT			
*Cla*μ2a	CCTTCAGGACCCACTTTCTT	58	30	23
*Cla*μ2b	CGAATCCCTCTTCCCAAACT			
*Cla*μ5a	GCGTAGACAGATGCATGGAA	55	30	22
*Cla*μ5b	CAGTCTGAAGCTAGGGCAAA			
*Cla*μ6a	GAAATATGGGACAGGGAGGA	58	30	15
*Cla*μ6b	GGTTGGCTGCATGTGTATGT			
*Cla*μ7a	CGGGGTCTTGGTGTTGACTA	58	30	13
*Cla*μ7b	CGGGACCAGGAGCTGTATAA			
*Cla*μ8a	CAGCCACTGAAGGAATTGAC	55	30	17
*Cla*μ8b	CACATACCTGACCCAGCTTATC			
*Cla*μ9a	ACAGGGGAAAAGAAGAGCTG	60	35	21
*Cla*μ9b	AAAATCCCCCACTCTTACCC			
*Cla*μ10a	TGGTCTTCTCTTCGTGTCCT	60	35	25
*Cla*μ10b	ATGAGCCCCTCTATGTTCCT			

PCR, polymerase chain reaction.

The RSTcalc package (Goodman, '97) was used to calculate ρ, an unbiased estimator of Slatkin's R_{ST} that corrects for potential biases that may result from unequal sample sizes and loci with unequal variances. R_{ST} statistics were estimated

with 10,000 permutations and 1,000 randomizations for the populations. R_{ST} estimates are more appropriate for loci analyses with high mutation rates, such as microsatellites (Slatkin, '95).

Nonrelated individuals from the captive colony of the species at the University of São Paulo were compared by ρ statistics with individuals from wild populations in order to check whether that colony represents well the genetic diversity of the species in Brazil. This genetic differentiation pattern was determined by neighbor-joining trees in PAUP version 4.0d63 (Swofford, '98). The correlation between genetic differentiation and geographic distance matrices was estimated by Mantel Test (software NTSYS-pc 1.70; Rohlf, '92).

RESULTS

Genetic diversity and heterozygosity

Analysis of the 11 markers resulted in the identification of 184 alleles (mean = 16.7, min = 3, max = 25; Table 2). The number of alleles per population varied from 40 to 74 (Table 1) with 50 exclusive alleles among all populations. Thirty (60%) of these presented frequency greater than 5%. Exclusive alleles with the lowest frequency (2%) were 211 and 243 in locus $Cla\mu2$ and 127 and 131 in locus $Cla\mu6$ in animals from Alagoas. The most frequent exclusive allele (100%) was 111 in locus $Ami\mu8$ in Bonito. Populations from Cardoso Island, Alagoas and Pantanal had most (52%) of the exclusive alleles (respectively 10, 9 and 8 alleles; Fig. 2).

The captive colony of the species at the University of São Paulo had a greater genetic diversity ($H_e = 0.738$) than the wild populations sampled ($0.563 < H_e < 0.673$, respectively, from Rio Grande do Sul and Charqueada). This was predictable as the captive colony founders came from different populations. The captive colony has a high genetic
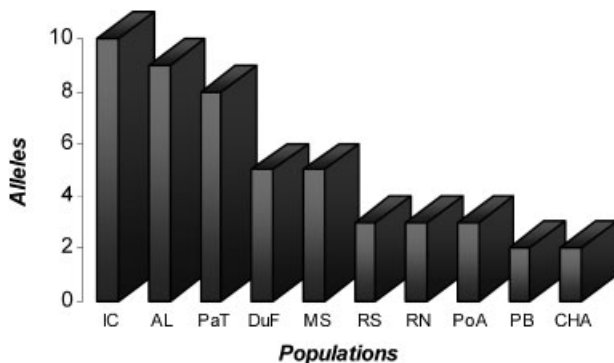
diversity and heterozygosity, which is fortunate for its farming program (Verdade, 2001b). However, it does not seem to efficiently represent the whole species for a possible ex situ conservation program.

Heterozygosity (H_o) varied from 0.444 (Alagoas) to 0.678 (Porto de Areia) with an average of 0.559 for all wild populations, which is similar to the A. mississippiensis ($H_o = 0.547$, according to Davis et al., 2002; $H_o = 0.570$, according to Ryberg et al., 2002). As heterozygosity is lower than genetic diversity, there are a large number of homozygotes. The overall F_{IS} value was 0.135, indicating a strong departure from panmixia, varying from 0.095 (Rio Grande do Sul) to 0.233 (Cardoso Island; Table 3).

Only Rio Grande do Norte and Cardoso Island did not differ significantly from HWE by Fisher Exact Test ($P > 0.05$) for all loci. The other populations showed significant deviation for at least one locus ($P < 0.05$), but no population deviated for all loci (Table 3). In most cases when a loci deviated from HWE (i.e., $H_e > H_o$), an excess of heterozygotes ($P \leq 0.01$) was found for the following markers: $Ami\mu11$ (Alagoas, Rio Grande do Sul, Cardoso Island, Rio Grande do Norte, Porto de Areia, Duraflora, Bonito and the captive colony at the University of São Paulo), $Cla\mu7$ (PaT) and $Cla\mu9$ (Duraflora and Cardoso Island).

Population genetic structure

Most of the genetic variation found (64.8%) was interpopulational, whereas only 35.2% was intrapopulational ($R_{ST} = 0.352$; $F_{ST} = 0.271$; $P < 0.001$; Table 4). This suggests that the populations sampled in this study are genetically well structured. Rho estimates statistically differed from 0 for all pairwise comparisons (Table 5) with the exception of Rio Grande do Norte and Paraíba ($\rho = -0.007$). On the other hand, Cardoso Island was the least related population to all the others, although it is approximately 300 km distant from the mainland populations sampled in the state of São Paulo (Duraflora, Porto de Areia and Charqueada) and the Pantanal.

DISCUSSION

The DNA microsatellite markers utilized in this study revealed moderate levels of polymorphism in populations of C. latirostris. Our estimates of F_{ST} and R_{ST} were statistically higher than zero for each comparison, suggesting a process of population subdivision, except for Paraíba and Rio

Fig. 2. Number of exclusive alleles per population.

TABLE 3. Statistical summary of microsatellite loci across all population of Caiman latirostris

Locus	AL	RS	IC	RN	PB	PoA	PaT	CH	DuF	MS	CAT	Mean
Amiμ8												
H_e	0.040		0.111	0.467	0.518	0.366	0.431	0.522	0.043		0.485	0.271
H_o	0.040**		0.111	0.200	0.250	0.000	0.400	0.333	0.043		0.667	0.186
F	0.000	Fixed	0.000	0.600	0.529	1.000*	0.073	0.371	0.000	Fixed	−0.429	0.329
Amiμ11												
H_e	0.691	0.515	0.686	0.756	0.373	0.660	0.606	0.659	0.730	0.763	0.545	0.635
H_o	1.000	1.000**	0.889	0.800	0.250	1.000*	0.450	0.333**	0.913**	0.900	1.000	0.776
F	−0.460	−1.000	−0.320	−0.067	0.340	−0.565	0.263	0.506	−0.257	−0.191	−1.000	−0.236
Amiμ13												
H_e	0.358	0.788	0.725	0.933	0.804	0.366	0.705	0.862	0.533	0.816	0.909	0.709
H_o	0.320	0.647	0.556	0.600	0.667*	0.444	0.700	0.667*	0.391	0.400**	0.667	0.551
f	0.107	0.183	0.245	0.385	0.178	−0.231	0.007	0.235	0.271	0.523	0.286	0.231
Amiμ20												
H_e	0.356	0.643	0.294	0.822	0.609	0.771	0.777	0.805	0.706	0.779	0.773	0.667
H_o	0.240	0.588	0.111	1.000	0.417	0.667**	0.526**	0.727	0.409**	0.700	0.667	0.550
f	0.330	0.088	0.636	−0.250	0.325	0.143	0.328	0.101	0.426	0.106	0.149	0.187
Claμ2												
H_e	0.823	0.401	0.758	0.844	0.848	0.529	0.591	0.656	0.344	0.753	0.803	0.668
H_o	0.360**	0.294	0.556	0.600	0.667	0.111	0.250**	0.250**	0.348	0.600	0.500	0.412
f	0.568	0.273	0.279	0.314	0.221	0.800	0.583	0.629	−0.011	0.212	0.400	0.397
Claμ5												
H_e	0.769	0.619	0.719	0.778	0.793	0.725	0.603	0.377	0.613	0.700	0.955	0.695
H_o	0.640**	0.824	0.667	0.600	0.833	1.000	0.600*	0.250*	0.591	0.700	1.000	0.700
f	0.171	−0.345	0.077	0.250	−0.053	−0.412	0.004	0.347	0.037	0.000	−0.053	−0.008
Claμ6												
H_e	0.716	0.433	0.523	0.356	0.083	0.699	0.637	0.717	0.635	0.753	0.621	0.561
H_o	0.240**	0.235**	0.444	0.400	0.083	0.556	0.250**	0.667*	0.348**	0.500*	0.167*	0.354
f	0.669	0.464	0.158	−0.143	0.000	0.216	0.614	0.074	0.458	0.348	0.750	0.385
Claμ7												
H_e	0.627	0.604	0.209	0.533	0.518	0.824	0.594	0.703	0.560	0.521	0.636	0.575
H_o	0.400*	0.294**	0.000	0.400	0.583	0.889	0.750**	0.833	0.391	0.100*	0.667	0.483
f	0.367	0.521	1.000	0.273	−0.132	−0.085	−0.272	−0.196	0.306	0.816	−0.053	0.169
Claμ8												
H_e	0.631	0.652	0.712	0.511	0.431	0.667	0.765	0.812	0.652	0.732	0.924	0.681
H_o	0.560	0.235**	0.444	0.200	0.417	1.000	0.650*	1.000	0.696	0.700	0.833*	0.612
f	0.115	0.646	0.390	0.636	0.035	−0.556	0.154	−0.245	−0.068	0.045	0.107	0.105
Claμ9												
H_e	0.872	0.893	0.771	0.889	0.819	0.529	0.613	0.627	0.766	0.779	0.712	0.752
H_o	0.600	0.706**	0.778	1.000	0.750**	1.000*	0.750*	0.750	0.957**	0.400*	0.667	0.760
f	0.316	0.215	−0.009	−0.143	0.088	−1.000	−0.231	−0.207	−0.256	0.500	0.070	−0.010
Claμ10												
H_e	0.622	0.649	0.869	0.511	0.623	0.758	0.767	0.667	0.793	0.889	0.758	0.719
H_o	0.480**	0.647*	0.778*	0.600	0.500	0.889	1.000*	0.917*	1.000*	0.900	0.667	0.762
f	0.232	0.003	0.111	−0.200	0.205	−0.185	−0.315	−0.399	−0.268	−0.013	0.130	−0.064
Mean												
H_e	0.591	0.563	0.580	0.673	0.584	0.627	0.644	0.673	0.580	0.680	0.738	0.630
H_o	0.444	0.497	0.485	0.582	0.492	0.687	0.575	0.612	0.553	0.536	0.682	0.559
f	0.220	0.095	0.233	0.150	0.158	−0.875	0.110	0.111	0.058	0.213	0.032	0.135

H_e, expected heterozygosity; H_o, observed heterozygosity; f, fixation index and exact test for Hardy–Weinberg equilibrium (* ≤ 0.05; ** ≤ 0.01).

Grande do Norte. In this case, a high genetic flux seems to occur, which is corroborated by the small geographic distance between these two locations (approximately 160 km).

The relatively high number of homozygotes found in this study suggests the occurrence of endogamy and/or genetic drift with inbreeding possibly caused by fragmentation of the species habitat owing to anthropogenic pressure. Similar results have been described for this species on a microgeographic scale by Verdade et al. (2002). However, for the American alligator this pattern

seems to occur only on a macrogeographic scale, where close populations (from Florida and Georgia, as well as from Texas and Louisiana) are genetically more similar (respectively, $R_{ST} = 0.032$ and $F_{ST} = 0.045$, and $R_{ST} = 0.040$ and $F_{ST} = 0.024$, according to Davis et al., 2002) than distant populations (Rockefeller Wildlife Refuge in Louisiana and Everglades National Park in Florida; $R_{ST} = 0.387$ and $F_{ST} = 0.137$, according to Glenn et al., '98).

Alleles become exclusive in wild populations owing to genetic isolation, mutation or natural selection (Futuyma, '98). Exclusive alleles can be useful in forensic issues such as the identification of the region of origin (not only the species in question) of wild specimens or their parts such as meat or skin. In addition, the occurrence of exclusive alleles stresses the importance of conservation of local populations.

Although located at the extremes of the species' geographic distribution, the populations from Rio Grande do Sul and northeastern Brazil (Alagoas, Paraíba and Rio Grande do Norte) are surprisingly more related to each other than to other populations from intermediate latitudinal ranges (Fig. 3). This can be owing to the fact that sea level was considerably lower along the southern and the eastern Brazilian coast during the Pleistocene epoch 16,000 years ago (Schwarzbold and Schafer, '84). During that period of lower sea levels, watercourses along the Brazilian coast were presumably connected thereby forming a vast coastal drainage area (Weitzman et al., '88) where genetic flux among broad-snouted caiman populations could occur without significant geographic barriers. The broad-snouted caiman population from Cardoso Island seems to be isolated from inland populations of the species regardless of the relatively small geographic distance between them (Table 5). The results suggest that the Atlantic Chain (*Serra do Mar*) is an effective geographic barrier between coastal and inland populations of the species, at least within the state of São Paulo, where the Atlantic Plateau can reach more than 1,000 m of altitude. The species is not usually found above 800 m (Yanosky, '94), which seems to corroborate this hypothesis. The channel between Cardoso Island and the continent is approximately 1.5 km at its narrowest point, which does not seem to be an effective geographic barrier, as the species seems to be able to move along relatively large distances of brackish water (Grigg et al., '98). However, the channel is composed by salt water. The broad-snouted caiman is a rather paludal species that seems to avoid large channels of open water (Medem, '83; Verdade, '98). In order to check how effective as a barrier this canal is, future studies should include animals from the local coastal drainage area.

There was no significant correlation between genetic variation and geographic distance considering

TABLE 4. Rho values over all populations

Locus	SA (across)	SW (within)	RHO (among)
$Ami\mu08$	0.86188	0.27991	0.75485
$Ami\mu11$	0.05756	1.01457	0.05368
$Ami\mu13$	0.15913	1.06923	0.12954
$Ami\mu20$	0.10625	0.81289	0.11559
$Cla\mu2$	0.61548	0.46988	0.56708
$Cla\mu5$	0.00324	1.42076	0.00227
$Cla\mu6$	0.39867	0.54613	0.42196
$Cla\mu7$	0.45712	0.59441	0.43472
$Cla\mu8$	0.22245	0.76160	0.22606
$Cla\mu9$	0.66277	0.38454	0.63283
$Cla\mu10$	0.50447	0.44137	0.53336
Average			0.35200

Number of permutations = 10,000.

TABLE 5. Rho values (overall average of loci in lower diagonal) and geographic distances in km (upper diagonal)

	AL	CH	DuF	MS	PoA	PB	PaT	RN	RS	IC
AL	–	1,840	1,912	2,474	1,866	365	1,851	467	2,986	2,062
CH	0.3751	–	110	922	26	2,197	12	2,299	1,199	284
DuF	0.4838	0.1013	–	816	95	2,266	105	2,363	1,170	*307*
MS	0.3779	0.3965	0.3687	–	913	2,812	920	2,869	1,310	998
PoA	0.4567	0.1670	0.1120	0.3795	–	2,223	15	2,325	1,175	247
PB	0.4050	0.3233	0.4281	0.4386	0.3589	–	2,209	**157**	3,352	2,425
PaT	0.4383	0.1269	0.2237	0.4501	0.1917	0.3785	–	2,311	1,187	273
RN	0.2254	0.3431	0.4375	0.4089	0.3642	**−0.007**	0.3670	–	3,466	2,535
RS	0.2596	0.2844	0.3859	0.4094	0.3380	0.2226	0.2890	0.2178	–	935
IC	0.1937	0.4939	*0.5642*	0.4373	0.5537	0.4798	0.4500	0.4719	0.4740	–

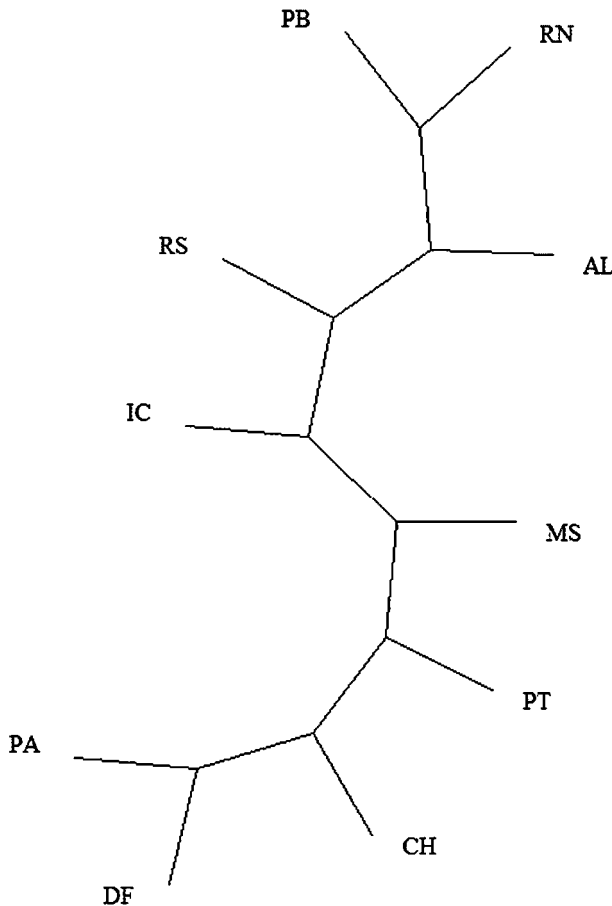Bold and italic numbers represent the lowest and highest genetic differentiations, respectively.

Fig. 3. Genetic differentiation pattern (R_{ST}) among populations (neighbor-joining method).

Fig. 4. Relationship between geographic distance and genetic differentiation ρ (squared). Triangles represent populations from northeastern and southern Brazil (AL-RS, PB-RS, RN-RS). Circles represent the relationship between populations on Cardoso Island and the continent. The log model is based on the squares, including all continental populations, except those represented by triangles.

all populations (Mantel Test: $r = 0.236$, $P = 0.112$). However, when Cardoso Island is excluded, such correlation becomes significant ($r = 0.476$, $P = 0.011$). In this case, an asymptotic model efficiently describes the relationship between genetic differentiation (ρ) and geographic distance, with the maximum rate of increase at approximately 280 km and asymptote at approximately 4,000 km (Fig. 4). The linear model can be expressed as: $\rho = 0.06 \, \mathrm{Ln}(x)0.0694$; $r^2 - 0.604$; $P < 0.001$, where $\mathrm{Ln}(x)$ = natural logarithm-transformed distance (km).

The relatively strong relationship between genetic differentiation and geographic distance (after excluding Cardoso Island and the coastal populations from the model) suggests a possible spatial scale for populations in genetic terms in which genetic flux seems to be minimal for distances greater than 4,000 km. However, this is close to the whole latitudinal range of the species and, as long as there was a river drainage covering this range, there seems to have been a consistent
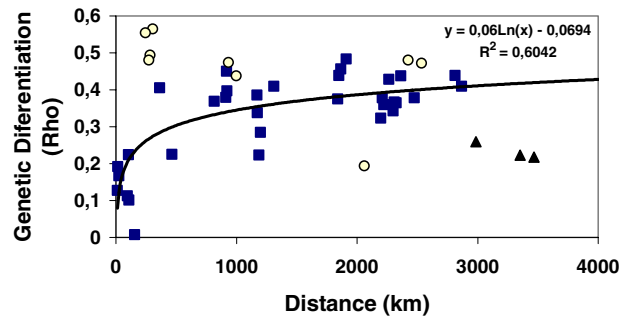
genetic flux throughout. On the other hand, continental populations of the species are spread over two main river basins: São Francisco and Paraná. The former runs northward, whereas the latter runs southward. The present results seem, therefore, more associated with macrogeographic patterns of the big river basins than geographic distances per se. Crocodilians use watercourses as their main pathway for dispersal (Magnusson, '79; Kay, 2004). The present results suggest that even large distances (thousands of kilometers) do not prevent genetic flux from occurring.

The maximum rate of increase in genetic differentiation occurs at a distance of 280 km for continental populations of the species. This distance is possibly related to the species' dispersal pattern (Caughley and Sinclair, '94; Sinclair et al., 2006) and individual's movement ability (Campos et al., 2006). These patterns should be considered in species conservation as long as no geographic barriers are involved.

In microgeographical terms, although the species seems to be able to colonize anthropogenetic habitats such as small artificial reservoirs in cattle ranches (Scott et al., '90) and build nests on pine (an exotic tree introduced to South America) (Verdade and Lavorenti, '90), its dispersal can be restricted by such circumstances (Verdade et al., 2002). This might lead to population fragmentation, genetic drift and inbreeding (Foose and Ballou, '88).

On a macrogeographic scale, the species' current distribution covers three major hydrographic basins: São Francisco, Paraná and small coastal drainages from Rio Grande do Sul to Rio Grande do Norte (Verdade and Piña, 2006). As these areas

coincide with the highest human population densities in South America, they all have problems of pollution, habitat loss and poaching. Small coastal drainages inhabited by the species in eastern Brazil are currently fragmented. However, in recent geological times they formed a large river drainage area with no apparent barrier for genetic flux of the species, which was relatively isolated from continental river basins (Schwarzbold and Schafer, '84). For this reason, they should also be considered for conservation purposes.

To date most studies of the species have been carried out in the Paraná River Basin in Argentina, Paraguay, Uruguay and southern Brazil (for a review, see Verdade and Piña, 2006). Present results suggest that the only population of broad-snouted caiman found in Paraguay River Basins (Bonito/Mato Grosso do Sul) is relatively isolated from the others and consequently warrant conservation efforts. In addition, future genetic studies of the species should include populations from the São Francisco River Basin. This little known region covers an extensive portion of the northern area of the species distribution (Verdade and Piña, 2006).

The captive breeding program for the species in São Paulo seems to have been effective in establishing a farming system in southeastern Brazil (Verdade, 2001b). Nevertheless, its possible value for ex situ conservation efforts should not be considered as an alternative for in situ conservation programs throughout the species' range. The broad-snouted caiman covers a large latitudinal area that can lead to varying selective pressures and genetic responses. The genetic structure of the species seems to be compatible with it.

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