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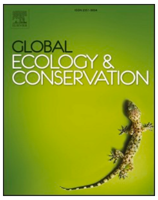

Effective population size of broad-snouted caiman (*Caiman latirostris*) in Brazil: A historical and spatial perspective

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ABSTRACT

Caiman latirostris has a large geographic distribution, that includes Argentina, Bolivia, Brazil, Paraguay, and Uruguay. In Brazil illegal hunting and land use change have caused population decline, relatively well documented in the last three decades. Due to such circumstances, the estimate of species effective population size might help analyze its viability. Single-sample estimator was used to estimate current effective population size (*Ne*) of broad-snouted caiman populations in representative areas of the species range in Brazil. For the analyzes, genotypes previously obtained were used for subpopulations of the captive colony of the University of São Paulo (USP) and for wild subpopulations. The microsatellites used were Amiμ8, Amiμ11, Amiμ13, Amiμ20, Claμ2, Claμ5, Claμ6, Claμ7, Claμ8, Claμ9 and Claμ10. The 11 loci analyzed produced 18.27 alleles on average. Wild populations showed significant genic and genotypic differentiation among them ($p < 0.01$). Population structure analyses (Rho-statistics) at Genepop for all loci was 0.376. The effective number of population sizes (Θ, the *Ne* estimator of Migrate-n software) of *Caiman latirostris* populations from the captive colony at the USP was 117.1 ($N = 7$) for NRF, 115.2 ($N = 4$) for Fm1, and 107.5 ($N = 4$) for Fm5, on the oposite side, the mean value of theta (Θ) was 7.7 ($N = 51$) for the Atlantic SE basin wild population, 9.4 ($N = 42$) for the Atlantic N/NE basin wild population and 9.6 ($N = 91$) for the Paraná basin wild population. The mean number of migrants (M of Migrate-n software) varied from 1.7 to 1.9 estimated by migrate-n are comparable to 1.3 estimated by Genepop. It is observed that in most wild population only few adults effectively contribute to genetic variation. The following guidlines are proposed as management

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actions: (1) an update of population sampling; (2) redefinition, delimitation and conservation of natural habitats; and (3) new conservation genetic studies with special attention to genetic diversity recovery.

1. Introduction

1.1. Geographic distribution

Caiman latirostris (Fig. 1) has a large geographic distribution, including Argentina, Bolivia, Paraguay, Uruguay and Brazil comprising the coastal basins from Rio Grande do Norte to Rio Grande do Sul and Paraná and São Francisco river basins (Nowell and Jackson, 1996; Verdade and Piña, 2006; Coutinho et al., 2013; Siroski et al., 2020), In Brazil its range includes the States of Rio Grande do Sul (Villela, 2004, 2009), Santa Catarina (Fusco-Costa et al., 2008), Paraná (Mourão and Campos, 1995), São Paulo (Mateos et al., 2002; Merege, 2009; Marques et al., 2016), Rio de Janeiro (Freitas Filho, 2008), Minas Gerais (Bertoluci et al., 2009), Espírito Santo (Silva-Soares et al., 2011), Alagoas (Moura, 2006; Gonçalves et al., 2012), Pernambuco (Mascarenhas-Júnior et al., 2020), Paraíba (Santana et al., 2008; Souto et al., 2011; de Souza, 2013), and Rio Grande do Norte (Verdade, 2001; Villela, 2004, 2009). In addition, the species distribution also includes parts of Sergipe, Bahia, and Goiás (Verdade and Piña, 2006).

The northernmost location of the species is Natal municipality in the state of Rio Grande do Norte (Verdade, 2001) and the southernmost is Taim in the state of Rio Grande do Sul (Melo, 2002). The westernmost population of the species in Brazil is Bonito, located in the state of Mato Grosso do Sul, and the easternmost is João Pessoa, located in the state of Paraiba (Villela, 2004, 2009).

C. latirostris is listed as Appendix I by CITES (CITES, 2021), except for Argentine populations (Prado et al., 2012). In addition, it is considered as Least Concern (LC) by the IUCN Red List (Siroski et al., 2020) and the Ministry of the Environment of Brazil (Coutinho et al., 2013; Brasil, 2014) and in São Paulo State (Bressan et al., 2009). The species is not included in the Red List in the Brazilian states of Rio Grande do Sul (SEMA, 2014), Paraná (Paraná, 2004; Tossulino et al., 2006), Santa Catarina (CONSEMA, 2011) and Minas Gerais (Minas Gerais, 2008; Passos et al., 2014). However, it is on the Red List of Bolivia (Aguirre et al., 2009; Rodriguez-Cordero et al., 2019).

Information about the conservation status of the broad-snouted caiman is scarce (Sabino and Prado, 2003), but the species is still illegally hunted in northeastern Brazil (Verdade, 2001; Alves et al., 2012). Relative abundance estimates across the broad-snouted caiman's distribution ranged from 0.07 to 58 ind/km, (Marques, 2013; Mourão and Campos, 1995; Pacheco and Llobet-Querejazu, 1998) (Table 1).

There is a paucity of large animals (Classes III and IV), attributed to hunting pressure in wild populations of the species in human-altered environments (Marques et al., 2016). A similar pattern has been described in the Três Marias Hydroelectric Reservoir (Passos et al., 2014), in southeast Brazil. However, this may be related to shy behavior as a response to hunting pressure (Verdade, 1996).

Fig. 1. Broad-snouted caiman (*Caiman latirostris*). Photo taken by Aloísio Cardoso at APA Gruta dos Brejões: Veredas do Romão Gramacho, Bahia, Brazil and reproduced with permission of the author.

1.2. Conservation genetics

Conservation genetics has been developed in the last three decades to deal with biological conservation using genetic tools (Ogden et al., 2009). Molecular markers have been applied to conservation genetic studies of *Caiman latirostris* to carry out parentage analysis in captive populations and assess dispersion patterns in natural populations, based on the application of microsatellite markers from *Alligator mississippiensis* producing the original results of Zucoloto (1998). Since then, other topics have been developed with the species (Amavet and Zucoloto, 2021; Amavet et al., 2021; Zucoloto et al., 2021), including a comprehensive review of the applicability of molecular markers including isozymes; RAPD and quantitative trait loci for the species in Argentina have also been presented (Amavet et al., 2010) (Table 2).

1.2.1. Micro and macrogeographic variation of Brazilian populations

The effects of habitat fragmentation on dispersal and mating system were studied in wild populations of the broad-snouted caiman of the eastern central region of São Paulo State (Verdade et al., 2002). In this study the authors concluded that there was a low number of individuals that effectively dispersed per generation among the various populations of the species (1.09 on average per generation).

Populations evaluated by these authors were composed predominantly of families, suggesting the scenario of local extinction, followed by recolonization predicted for fragmented populations by metapopulation approaches. Microsatellite markers have been developed to assess the coefficient of kinship between individuals and to study dispersal patterns among wild populations (Zucoloto, 1998, 2003; Amavet et al., 2017). It was observed that individuals from three geographically close groups in nature were barely mixed, indicating that this method could be used in conjunction with F statistics to evaluate the structure of wild populations of broad-snouted caiman and its dispersal patterns and mechanisms. In fact, a high level of structuring both at micro and macrogeographic scale is due to the diversity between local populations composed of endogamic groups (Zucoloto, 1998, 2003; Verdade et al., 2002; Villela, 2004, 2009).

1.2.2. Age distribution and mating system

Age distribution is usually biased towards hatchlings and young, with subadults and adults less common (Borteiro et al., 2008; Merege, 2009; Passos et al., 2014; Marques et al., 2016). In addition, parental care has been exhibited by non-reproductive females over viable nests of shy females in captivity in Brazil (Zucoloto, 1998, 2003; Zucoloto et al., 2009). Multiple clutches (Larriera, 2002) and multiple paternity (i.e., polyandry) (Amavet et al., 2008, 2010, 2012, 2015; Amavet, 2009) have been observed in wild populations in Argentina. Polyandry has also been observed in other crocodilians (Davis et al., 2001; McVay et al., 2008; Lance et al., 2009; Oliveira, 2010; Oliveira et al., 2010, 2014; Muniz et al., 2011; Zajdel et al., 2019; Isberg, 2021).

1.2.3. Effective population size

Recently a single-sample estimator of contemporary effective population size (*Ne*) has been developed, its advantages over other methods have been discussed by its authors (Do et al., 2014). This method is based on the implementation of the estimator developed by Nomura (2008) to assess the number of actual breeders based on the molecular demonstration of common ancestry of a single cohort sample. Another effective implementation of algorithms to estimate effective population size and migration includes a coalescence method using Maximum Likelihood or Bayesian analysis as a criterion (Beerli, 2006; Beerli and Felsenstein, 2001; Beerli and Palczewski, 2010).

Considering the previous results addressing the molecular ecology and conservation genetics of *Caiman latirostris*, the present study aimed to estimate the effective number of breeders and migration of Brazilian populations of broad-snouted caiman based on single samples. Our hypothesis is that the general pattern of relatively low effective population size and gene flow (as described at local levels by Verdade et al., 2002 and Zucoloto, 2003) also occurs at the regional level in Brazil. Such information might be critical to understanding the species' conservation genetics.

Table 1
Relative abundance of the specie.

Relative abundance (ind/km)	Site	References
0.07	Silvicultural landscapes of São Paulo	Marques, 2013
11.3	Silvicultural landscapes of São Paulo	Marques et al., 2016
0.07–0.28	Paraná River	Mourão and Campos, 1995
0.25	Northwestern area of Santa Catarina Island	Fusco-Costa et al., 2008
< 1	Grande Sertão Veredas National Park	Carvalho and Batista 2013
6–8	Northeastern Brazil	Verdade, 2001
2.1–4.4	Altered landscapes in northwestern Uruguay	Borteiro et al., 2008
8	Alagoas	Coutinho et al., 2013
3–58	Pilcomayo River basin in Bolivia that is the highest relative abundance reported for the species	Pacheco and Llobet-Querejazu 1998
0.3-3.8	Três Marias Hydroelectric Reservoir	Mascarenhas-Júnior et al., 2020

Table 2
Themes of conservation genetics studies carried out with *Caiman latirostris*.

Theme	Reference	Observation
Isolation of new microsatellite markers for the species	Zucoloto, 2003; Amavet et al., 2015	
Cross-species marker transference	Miles et al., 2009	From *Crocodylus porosus* to *Caiman crocodilus*, *Caiman latirostris*, *Caiman yacare*, *Melanosuchus niger* and *Paleosuchus palpebrosus*
	Zucoloto et al., 2006	For other Brazilian crocodilians: from *Caiman latirostris* to *Caiman crocodilus*, *Caiman yacare* and *Paleosuchus palpebrosus*
	Amavet et al., 2015	From *Caiman latirostris* to *Caiman yacare*
Parentage analysis and mating system	Zucoloto, 1998, 2003; Amavet et al., 2008, 2012; Zucoloto et al., 2009	
Population genetic diversity and structure	Zucoloto, 1998, 2003; Verdade et al., 2002	Microgeographic variation
	Villela, 2004, 2009; Villela et al., 2008; Amavet et al., 2012	Macrogeographic variation
Phylogeny studies used mitochondrial and nuclear markers	Hrbek et al., 2008; Villela 2009	
Review of molecular markers use	Amavet et al., 2010	

Table 3
A comparison of the captive population at the University of Sao Paulo and Wild populations.

Captive population[a]	Sub-population	n	Origin	Observation
Colony of USP	1 - NRF	7	22°45’ latitude S, 47°39’ longitude W	Non related founders of captive colony of USP
Colony of USP	2 – Fm1	4	22°45’ latitude S, 47°39’ longitude W	Hatchlings from family 1 of captive colony of USP
Colony of USP	3 – Fm5	4	22°45’ latitude S, 47°39’ longitude W	Hatchlings from family 5 of captive colony of USP
Wild population[b]	**Sub-Population**	**n**	**Origin**	**Observation**
F	4 – PA[c]	9	22°39’ latitude S, 47°58’ longitude W	Porto de Areia, a lake at Paraná river basin
F	5 – VG[c]	2	22°40’ latitude S, 47°55’ longitude W	Volta Grande, is a wetland at Paraná river basin
F	6 – PT[c]	20	22°35’ latitude S, 47°55’ longitude W	Pantanal, is a wetland at Paraná river basin
F	7 – CH[c]	12	22°30’ latitude S, 47°48’ longitude W	Charqueada, is an artificial reservoir in a cattle ranch at Paraná river basin
F	8 – DF[c]	23	22°26’ latitude S, 48°52’ longitude W	Duraflora, is an artificial lake in eucalyptus plantations at Paraná river basin
F	9 – AN[c]	2	23°36’ latitude S, 48°26’ longitude W	Angatuba/SP, Ribeirao Jacu at Paraná river basin
F	10 – PR[c]	3	21°32’ latitude S, 51°36’ longitude W	Rio Paraná at Paraná river basin
F	11 – GR[c]	5	20°07’ latitude S, 48°54’ longitude W	Rio Grande at Paraná river basin
F	12 – JP[c]	4	22°01’ latitude S, 48°25’ longitude W	Rio Jacaré-pepira at Paraná river basin
F	13 – MS[c]	10	21°07’ latitude S, 56°30’ longitude W	Bonito/MS, is a weir at Paraná river basin
H	14– CB[d]	25	23°54’ latitude S, 46°25’ longitude W	Cubatao/SP, is a lagoon at Atlantic Southeast basin
H	15– IC[d]	9	25°04’ latitude S, 47°55’ longitude W	Ilha do Cardoso/SP, is a creek at Atlantic Southeast basin
H	16– RS[d]	17	32°32’ latitude S, 52°23’ longitude W	Rio Grande do Sul, is a wetland at Atlantic Southeast basin
C	17– RN[d]	15	5°43’ latitude S, 5°12’ longitude W	Rio Grande do Norte, is a wetland at Atlantic North/Northeast basin
C	18– PB[d]	12	7°06’ latitude S, 4°52’ longitude W	Paraiba, is a wetland at Atlantic North/Northeast basin
C	19– AL[d]	25	10°04’ latitude S, 6°21’ longitude W	Alagoas, is a lake at Atlantic North/Northeast basin

[a] Data and genotypes were from previous studies (Zucoloto, 1998, 2003; Verdade et al., 2002).

[b] Paraná river basin (F), Atlantic SE river basin (H) and Atlantic N/NE river basin (C).

[c] Data and genotypes were from previous studies (Zucoloto, 1998, 2003; Verdade et al., 2002).

[d] Data and genotypes were from previous studies (Villela, 2004, 2009; Villela et al., 2008).

2. Study area

A total of 198 individuals were analyzed from six populations, three of which were from a captive colony at the University of São Paulo (USP) and three populations from the wild (Table 3), at known geographic localization from previous studies (Zucoloto, 1998, 2003; Verdade et al., 2002; Villela, 2004, 2009; Villela et al., 2008). Fig. 2 shows the geographic localities on the map. This organization of wild populations follow the results of *Caiman latirostris* phylogeography, which shows three Evolutionary Significant Units (ESUs) associated with the hydrographic basins, based on mitochondrial (COI and 16sRNA) and nuclear (RAG1) gene sequence analyses (Borges et al., 2018).

3. Methodology

For the analyses, previously obtained genotypes were used (Zucoloto, 1998, 2003; Verdade et al., 2002) for subpopulations from the captive colony at the University of Sao Paulo (USP), Table 3; (Zucoloto, 1998, 2003; Verdade et al., 2002; Villela, 2004, 2009; Villela et al., 2008) and for wild subpopulations as indicated in Table 3. Below is a brief description of the methodology used at these above mentioned previous studies, from which the genotypes were used for the current analyzes of effective population sizes and migration among the studied populations.

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 (Walsh, 1987), and adults were noosed (Hutton et al., 1987). Captive animals were processed during daylight hours. Physical techniques were applied to immobilized animals without the use of tranquilizers (Huchzemeyer, 2003). Blood was collected by puncturing the dorsal branch of the superior cava vein (Olson et al., 1975). Collected blood sample was stored in lysis buffer (Hoelzel, 1992): 100 mM Tris–HCl, pH 8.0; 100mM EDTA, pH 8.0; 10mM NaCl; 0.5% SDS (pv^{-1}).

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 (Hoelzel, 1992; Olerup and Zetterquist, 1992). Eleven primer pairs were utilized, four (Amiμ8,

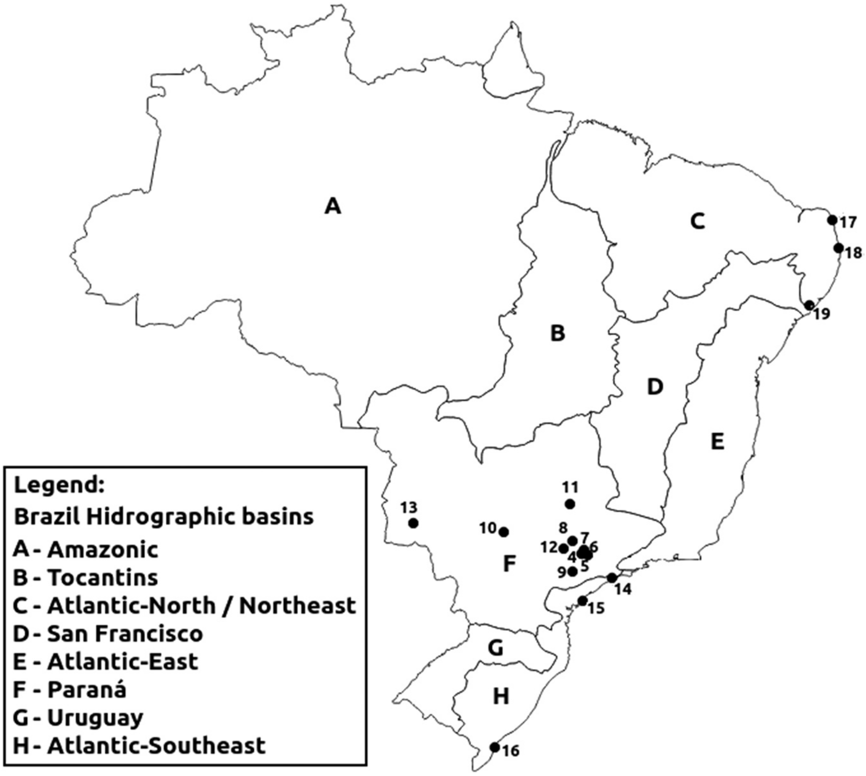

Fig. 2. Distribution of the broad-snouted caiman *(Caiman latirostris)* populations according hydrographic basins. 1 - NRF (Non related founders of captive colony of USP, n = 7), 2 – Fm1 (hatchlings from family 1 of captive colony of USP, n = 4), 3 – Fm5 (hatchlings from family 5 of captive colony of USP, n = 4) are not shown, 4 – PA (Porto de Areia, n = 9), 5 – VG (Volta Grande, n = 2), 6 – PT (Pantanal, n = 20), 7 – CH (Charqueada, n = 12), 8 – DF (Duraflora, n = 23), 9 – AN (Angatuba/SP, n = 2), 10 – PR (Rio Paraná, n = 3), 11 – GR (Rio Grande, n = 5), 12 - JP (Rio Jacarépepira, n = 4) and 13 – MS (Bonito/MS, n = 10) representing Paraná hydrographic basin; 14 – CB (Cubatao/SP, n = 25), 15 – IC (Ilha do Cardoso/SP, n = 9), and 16 – RS (Rio Grande do Sul, n = 17), collected from Atlantic-Southeast hydrographic basin; and 17 – RN (Rio Grande do Norte, n = 5), 18 – PB (Paraíba, n = 12) and 19 – AL (Alagoas, n = 25), located at Atlantic-North / Northeast hydrographic Basin.

Table 4

Genetic diversity measures and Hardy-Weinberg exact test for the loci tested.

*Ami*μ8	NRF	Fm1	Fm5	F	H	C	*Cla*μ6	NRF	Fm1	Fm5	F	H	C
N	**7**	**4**	**4**	**91**	**51**	**42**	***N***	**7**	**4**	**4**	**91**	**51**	**42**
He	3.077	2.143	1.714	47.173	5.752	12.470	**He**	5.769	1.714	2.286	67.704	20.604	31.386
Ho	4	3	2	14	4	5	**Ho**	3	2	4	39	13	9
Fis	-0.333	-0.500	-0.200	0.704	0.307	0.602	**Fis**	0.500	-0.200	-1	0.425	0.371	0.716
HW	1.000	1.000	1.000	0.000	0.144	0.001	**HW**	0.032	1.000	0.314	0.000	0.001	0.000
SE	-	-	-	-	-	-	**SE**	0.001	-	-	0.000	0.000	0.000
Sig.	ns	ns	ns	**	ns	**	**Sig.**	*	ns	ns	**	**	**
***Ami*μ11**	**NRF**	**Fm1**	**Fm5**	**F**	**H**	**C**	***Cla*μ7**	**NRF**	**Fm1**	**Fm5**	**F**	**H**	**C**
N	**7**	**4**	**4**	**91**	**51**	**42**	***N***	**7**	**4**	**4**	**91**	**51**	**42**
He	4.615	2.286	2.286	70.888	40.129	29.012	**He**	5.154	2.143	2.714	68.927	18.347	31.723
Ho	6	4	4	68	38	32	**Ho**	5	3	3	49	7	19
Fis	-0.333	-1.000	-1.000	0.041	0.054	-0.104	**Fis**	0.032	-0.500	-0.125	0.290	0.621	0.404
HW	0.007	0.314	0.314	0.000	0.000	0.003	**HW**	0.401	1.000	1.000	0.000	0.000	0.000
SE	-	-	-	0.000	0.000	0.001	**SE**	0.003	-	-	0.000	0.000	-
Sig.	**	ns	ns	**	**	**	**Sig.**	ns	ns	ns	**	**	**
***Ami*μ13**	**NRF**	**Fm1**	**Fm5**	**F**	**H**	**C**	***Cla*μ8**	**NRF**	**Fm1**	**Fm5**	**F**	**H**	**C**
N	**7**	**4**	**4**	**91**	**51**	**42**	***N***	**7**	**4**	**4**	**91**	**51**	**42**
He	6.538	3.143	2.714	77.436	43.010	25.337	**He**	6.615	3.143	3	76.379	38.753	30.072
Ho	5	4	1	49	32	19	**Ho**	7	4	4	71	24	20
Fis	0.250	-0.333	0.667	0.369	0.258	0.252	**Fis**	-0.063	-0.333	-0.412	0.071	0.383	0.338
HW	0.010	1.000	0.086	0.000	0.000	0.002	**HW**	1.000	0.057	1.000	0.000	0.000	0.002
SE	0.001	-	-	0.000	0.000	0.001	**SE**	0.000	-	-	0.000	0.000	0.000
Sig.	*	ns	ns	**	**	**	**Sig.**	ns	ns	ns	**	**	**
***Ami*μ20**	**NRF**	**Fm1**	**Fm5**	**F**	**H**	**C**	***Cla*μ9**	**NRF**	**Fm1**	**Fm5**	**F**	**H**	**C**
N	**7**	**4**	**4**	**91**	**51**	**42**	***N***	**7**	**4**	**4**	**91**	**51**	**42**
He	5.769	1	1	71.556	34.485	30.651	**He**	4.923	2.714	2.857	64.765	43.347	37.952
Ho	5	1	1	48	22	28	**Ho**	5	4	4	71	34	29
Fis	0.143	0.000	0.000	0.331	0.364	0.087	**Fis**	-0.017	-0.600	-0.500	-0.097	0.217	0.238
HW	0.289	-	-	0.000	0.000	0.009	**HW**	0.808	0.314	0.543	0.000	0.000	0.000
SE	0.006			0.000	0.000	0.001	**SE**	-	-	-	0.000	0.000	0.000
Sig.	ns	nt	nt	**	**	**	**Sig.**	ns	ns	ns	**	**	**
***Cla*μ2**	**NRF**	**Fm1**	**Fm5**	**F**	**H**	**C**	***Cla*μ10**	**NRF**	**Fm1**	**Fm5**	**F**	**H**	**C**
N	**6**	**4**	**4**	**91**	**51**	**42**	***N***	**7**	**4**	**4**	**91**	**51**	**42**
He	4.909	2.143	1.714	72.330	43.149	37.843	**He**	4.769	3.286	1.714	79.089	45.436	25.518
Ho	4	3	2	33	26	20	**Ho**	3	4	2	85	27	21
Fis	0.200	-0.500	-0.200	0.545	0.400	0.475	**Fis**	0.390	-0.263	-0.200	-0.075	0.408	0.178
HW	0.190	1.000	1.000	0.000	0.000	0.000	**HW**	0.069	0.771	1.000	0.000	0.000	0.009
SE	0.003	-	-	0.000	0.000	0.000	**SE**	0.002	-	-	0.000	0.000	0.001
Sig.	ns	ns	ns	**	**	**	**Sig.**	ns	ns	ns	**	**	**
***Cla*μ5**	**NRF**	**Fm1**	**Fm5**	**F**	**H**	**C**							
N	**7**	**4**	**4**	**91**	**51**	**42**							
He	6.615	2.714	2.857	72.554	39.574	35.783							
Ho	6	4	4	56	30	29							
Fis	-0.063	-0.600	-0.500	0.229	0.244	0.191							
HW	1	0.314	0.543	0.000	0.000	0.002							
SE	0	-	-	0.000	0.000	0.000							
Sig.	ns	ns	ns	**	**	**							

Subtitle: N - Number of individuals sampled, He - Number of heterozygous individuals expected according to the Hardy - Weinberg equilibrium, Ho - Number of heterozygous individuals observed, Fis - Fixation index of Weir & Cockerham (1994), HW - Exact test of adherence to the Hardy-Weinberg equilibrium, SE - Standard error of estimate of the probability of the exact test of adherence to the Hardy-Weinberg equilibrium, Sig. - Significance of the test, ns - Not significant, nt - Not tested, * ($p < 0.05$), ** ($p < 0.01$). Obs.: NRF (Nonrelated founders of captive colony of USP), 2 – Fm1 (hatchlings from family 1 of captive colony of USP), 3 – Fm5 (hatchlings from family 5 of captive colony of USP), Paraná basin (F), Atlantic SE basin (H) and Atlantic N/NE basin (C).

Amiμ11, Amiμ13 and Amiμ20) for *Alligator mississippiensis* (Glenn et al., 1998) and seven (Claμ2, Claμ5, Claμ6, Claμ7, Claμ8, Claμ9 and Claμ10) for *Caiman latirostris* (Zucoloto et al., 2002).

Amplification conditions were as follows: polymerase chain reaction (PCR) buffer (20 mM Tris–HCl, pH 8.4; 50 mM KCl), 1.5 mM $MgCl_2$, 0.2m M 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 mL. Amplification was as follows: (1) 94 °C for 3 min, (2) 94 °C for 45 sec, (3) primer pair annealing temperature for 1 min, (4) 73 °C for 1 min and 15 sec, (5) repeat steps (2), (3) and (4) n cycles (Zucoloto, 2003; Zucoloto et al., 2002), (6) 41 °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).

Descriptive statistics analysis of wild populations were performed using Genepop (Rousset, 2008). Populations were tested for adherence to Hardy-Weinberg (HW) equilibrium by exact test (Option 1, sub-option 3 with 10,000 dememorisations, 100 batches and 5000 interactions per batch). Genic differentiation for all pairs of wild populations (Option 3, sub-option 2 with 10,000 dememorisations, 100 batches and 5000 interactions per batch) and genotypic differentiation for all pairs of wild populations (Option 3, sub-option 4 with 10,000 dememorisations, 100 batches and 5000 interactions per batch) was also tested.

Diversity measures were also estimated (Option 5, sub-option 1). Nm estimates were also done in order to measure gene flow among wild populations to compare with results from migrate-n (Option 4). Rho-statistics were estimated among wild populations (Option 6, sub-option 3). The effective population size (Ө, the *Ne* estimator of Migrate-n software) and migration (M of Migrate-n software) among populations were estimated by the method of coalescence using Bayes analysis (Beerli, 2006; Beerli and Felsenstein, 2001; Beerli and Palczewski, 2010).

4. Results

The 11 loci analyzed produced 18.27 alleles on average, ranging from four alleles at locus *Ami*μ8 to 28 alleles at locus *Cla*μ5. Genetic diversity deviated from HW equilibrium in the captive colony of USP only at markers *Ami*μ11 (NRF, heterozygote excess), *Ami*μ13 (NRF, heterozygote deficiency), and *Cla*μ6 (NRF, heterozygote deficiency; Table 4). All wild populations presented heterozygote deficiencies, except for the population from Atlantic SE (marker *Ami*μ8 that was in HW equilibrium), Atlantic N/NE population with marker *Ami*μ11 (significant heterozygote excess), and Parana river basin with marker *Cla*μ9 and *Cla*μ10 (heterozygote excess; Table 4).

Wild populations showed significant genic and genotypic differentiation among them ($p < 0.01$), but this differentiation was not confirmed in analyses across all loci (Table 5). The results of population structure analyses at Genepop for all loci demonstrated a moderate to high level of population structuring (Rho(st) equal to 0.376) (Table 6).

The effective number of population sizes (Ө, the *Ne* estimator of Migrate-n software) of *Caiman latirostris* populations from the captive colony at the USP (NRF, Fm1 and Fm5), and of the populations from three Brazilian hydrographic basins (Paraná, Atlantic SE and Atlantic N/NE) were estimated by migrate-n software (Table 7). The mean value of theta (Ө) was 117.1 ($N = 7$) for NRF, 115.2 ($N = 4$) for Fm1, and 107.5 ($N = 4$) for Fm5. On the other hand, the mean value of theta (Ө) was 7.7 ($N = 51$) for the Atlantic SE basin wild population, 9.4 ($N = 42$) for the Atlantic N/NE basin wild population and 9.6 ($N = 91$) for the Paraná basin wild population.

The mean number of migrants (M) among the pairs of wild populations varied from 1.7 to 1.9. The low mean number of migrants among wild populations (1.7) was from the Atlantic SE basin to the Paraná basin; and the high (1.9) was from the Atlantic N/NE basin to the Paraná basin as estimated by migrate-n (Table 7). These mean numbers of migrants among wild populations are comparable to the mean number of migrants among this populations after correction for size (1.3) estimated by Genepop.

5. Discussion

The exact test for HW equilibrium and the measurements of heterozygosity showed, with few exceptions, that the population of the captive colony of the University of São Paulo was in HW equilibrium. In the wild, deviations of HW with heterozygote deficiency were observed in populations of the Paraná, Atlantic SE and Atlantic N/NE basins (with the exceptions previously mentioned). One hypothesis to explain such deviation could be the presence of events like genetic drift or inbreeding depression following a bottleneck (Table 4).

A genic differentiation among wild populations was observed at the test by population pair for each locus (Fisher's method) ($p < 0.01$). In fact, exclusive alleles can be observed in all these wild populations (Zucoloto, 2003: Table 5) and others that were previously reported (Villela, 2009).

Table 5
Genic and genotypic differentiation by population pair across all loci (Fisher's method).

Genic differentiation					Genotypic differentiation				
Pop pair	Chi 2	df	P-Value	Significance	Pop pair	Chi 2	df	P-Value	Significance
F - H	> 0.000	22	< 1.000	ns	F - H	> 0.000	22	< 1.000	ns
F - C	> 20.356	22	< 0.561	ns	F - C	> 10.136	22	< 0.985	ns
H - C	> 20.967	22	< 0.523	ns	H - C	> 29.604	22	< 0.128	ns

Subtitle: ns - Not significant. Obs.: Paraná basin (F), Atlantic SE basin (H) and Atlantic N/NE basin (C).

Table 6
Allele size-based covariance (RhoIS, RhoST, RhoIT).

Locus	Rho(is)	Rho(st)	Rho(it)
Amiµ8	0.843	0.008	0.844
Amiµ11	-0.246	0.213	0.020
Amiµ13	0.280	0.297	0.494
Amiµ20	0.218	0.004	0.222
Claµ2	0.708	0.255	0.783
Claµ5	0.295	0.125	0.383
Claµ6	0.284	0.465	0.617
Claµ7	0.199	0.437	0.549
Claµ8	0.210	0.273	0.426
Claµ9	0.395	0.638	0.781
Claµ10	0.324	0.312	0.535
All	0.308	0.376	0.568

Table 7
Overall loci bayesian estimates of effective population size (ϴ) and migration (M) of *Caiman latirostris* populations from three Brazilian hydrographic basins.

Locus	Parameter	2.5%	25.0%	Mode	75.0%	97.5%	Median	Mean
All	Theta (ϴ)_NRF	111.000	114.500	116.750	119.500	126.500	117.750	117.106
All	Theta (ϴ)_Fm1	106.500	113.000	116.750	119.500	123.500	116.250	115.169
All	Theta (ϴ)_Fm5	99.000	106.000	108.250	110.500	115.000	108.250	107.468
All	Theta (ϴ)_F	9.000	9.320	9.540	9.760	10.120	9.620	9.574
All	Theta (ϴ)_H	6.720	7.440	7.700	7.960	8.560	7.780	7.738
All	Theta (ϴ)_C	8.920	9.160	9.380	9.560	9.920	9.460	9.406
All	M_H->F	1.280	1.520	1.660	1.800	2.000	1.700	1.669
All	M_C->F	1.520	1.720	1.900	2.000	2.280	1.940	1.903
All	M_F->H	1.360	1.560	1.740	1.840	2.080	1.780	1.732
All	M_C->H	1.440	1.680	1.860	2.000	2.240	1.900	1.843
All	M_F->C	1.440	1.680	1.860	2.000	2.200	1.900	1.847
All	M_H->C	1.480	1.720	1.860	2.000	2.240	1.900	1.882

Obs.: NRF (Nonrelated founders of captive colony of USP), 2 – Fm1 (hatchlings from family 1 of captive colony of USP), 3 – Fm5 (hatchlings from family 5 of captive colony of USP), Paraná basin (F), Atlantic SE basin (H) and Atlantic N/NE basin (C).

The heterozygote deficiency revealed by the test of HW equilibrium (Table 4) for the wild populations was reflected by the differentiation among the wild populations from these major Brazilian hydrographic basins observed in the genotypic differentiation test by population pair for each locus (Fisher's method) ($p < 0.01$). This could be reflected in the level of population subdivision shown by Rho statistics (Table 6), because it tests for differentiation among populations. The Rho statistics (n unbiased estimator of Slatkin's Rst) demonstrate that there is subdivision among wild populations. The low mean number of migrants per generation among these populations can be attributed to this differentiation (Table 7).

The genic and genotypic differentiation observed in tests by population pair for each locus (Fisher's method) ($p < 0.01$), were not significant for the test of population pairs across all loci (Fisher's method, Table 5). See Ohta and Gillespie (1996) for further discussion of nearly neutral theory.

The average of effective numbers of population size estimate (ϴ), which is the number of breeders of a population estimated from genotypes, suggests that the captive colony of USP formed by individuals from diverse localities across Brazil (Verdade and Kassouf-Perina, 1993), retain a considerable amount of the genetic diversity among Brazilian populations of the species, which is reflected by its means of ϴ: 117.106 (N = 7) for NRF. As expected, the mean ϴ estimated from the offspring of two families of this captive colony was similar to the mean ϴ values estimated for NRF, 115.169 (N = 4) for Fm1 and 107.468 (N = 4) for Fm5.

On the other hand, the averages of ϴ for wild populations in Brazilian river basins reveals that the number of breeders that contributed to the formation of these wild populations was relatively small: 7.738 (N = 51) for Atlantic SE, 9.406 (N = 42) for Atlantic N/NE, and 9.574 (N = 91) for Parana river basin (Table 7). As previously reported the relative abundance of the species in Brazil is low, ranging from 0.07 to 11.3 Ind/Km (Table 1). The mean value of ϴ for wild populations should be analyzed as a critical warning sign for the conservation of the *Caiman latirostris* in Brazil, because it shows the likely relationship between low population densities and genetic diversity with a limited number of effective breeders.

A recent review of the South American vertebrate diversification shows that groups from these regions underwent a diverse event of climate and geological changes, modifying both continents and oceans and creating complex scenarios for speciation and intra-specific diversification. Turchetto-Zolet et al. (2013), point out that rivers could be barriers for terrestrial animals, although they can be the primary way for aquatic organisms to disperse.

Some crocodilian species can move long distances (> 150 km) from one hydrographic basin to another by the land, like the freshwater crocodile in Australia (Cao et al., 2020). *C. latirostris* is highly aquatic, dwelling on densely vegetated and quiet waters like marshes, swamps, and mangroves (Medem, 1983). Home range of the species was measured in silvicultural landscapes that was

96.6 ± 183.9 ha (males) and 43.2 ± 78.6 ha (females) estimated by Minimum Convex Polygon (MPC) and 95% Kernel Density Estimate methods (KDE) (Marques et al., 2020). Little is known about its dispersal patterns and use of space in either micro- or macrogeographic variation (Verdade et al., 2002; Villela et al., 2008). We can assume that the species uses the main water courses and their tributaries to disperse, much like the Nile crocodile (Hutton, 1989).

The present results suggest that the dispersal patterns of *C. latirostris* are relatively small. As a result, hydrographic basins they inhabit tend to be mostly isolated (Borges et al., 2018). Our results suggest that the movement of crocodilians through distinct hydrographic basins may be limited by barriers (as originally suggested by Fukuda et al., 2019). As a matter of fact, even small groups living within the same small drainage system are usually composed of isolated related individuals with RST values between 0.186 and 0.191 respectively, (Verdade et al., 2002; Zucoloto, 2003) compared with *Alligator mississippiensis* (RST = 0.387, according to Glenn et al., 1998). Considering that the age at sexual maturity of *Caiman latirostris* is between five and 10 years (Verdade and Sarkis, 1998; Verdade et al., 2003; Larriera et al., 2006), and the mean number of migrants estimated among these wild populations (1.333 per generation, by Genepop), such a pattern can lead to inbreeding depression. This is in accordance with the three Evolutionary Significant Units (ESUs) associated with the Brazilian hydrographic basins (Borges et al., 2018) and the macrogeographic genetic pattern described by Villela et al. (2008).

The release of young, captive reared *C. latirostris* by the Argentine ranching program has apparently increased gene flow and genetic variability ($Ho = 0.4415$; $He = 0.7455$) at the local population level after a possible bottleneck the species suffered on the past. The global F_{ST} value estimated by Amavet et al. (2017) was 0.224 ($P < 0.001$, CI 0.200-0.257. The *Ne* estimated for the Argentine population was 2.1 (Amavet et al., 2017), which can be considered low if it is compared with the Brazilian populations (9.574 for Parana river basin, 7.738 for Atlantic SE and 9.406 for Atlantic N/NE). However, the present results suggest that the number of breeders in Brazilian populations is lower than the *Ne* of the NRF population from USP (117.106; N = 7). Amavet et al. (2017) argue that this low value of *Ne* estimated for the Argentine population from the Paraná river basin is due to the overhunting that occurred during the last 70 years. However, the same situation may have occurred with the Brazilian population.

The actual Θ and mean number of migrants among pairs of wild populations, respectively, showed that there were a low number of breeders contributing to the overall genetic variation in these wild populations and little gene flow between populations. Such a pattern suggests that hydrographic basins work as pathways for dispersing individuals within the same basin but as barriers between distinct ones. For this reason, individual hydrographic basins should be considered Evolutionary Significant Units (ESUs) for the purpose of biological conservation of *Caiman latirostris* in Brazil.

At the next assessment of risk, the actual Siroski et al. (2020) classification of *Caiman latirostris* should consider our results of *Ne* (Θ) and M (Mean number of migrants among populations of hydrographic basins), at this case the classification of the species could be changed from Least Concern (LC) to a more conservative classification. Efforts should be concentrated at the San Francisco (D) and Atlantic East (E) hydrographic basins. To do so, the following recommendations should be considered: (1) an update of population sampling on the main river basins occupied by the species; (2) redefinition, delimitation and conservation of natural habitats for the conservation of the species in the wild; and (3) monitoring genetic diversity in wild populations along the species range.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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