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Caiman latirostris (Daudin, 1802) phylogeography and applications for its conservation

Vitor Souza Borges

Belo Horizonte

2014

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Dissertação apresentada ao Programa de Pós-graduação em Zoologia de Vertebrados da PUC Minas, como requisito parcial para obtenção do grau de Mestre em Zoologia.

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ABSTRACT

Phylogeography is the field that investigates geographical processes influencing genealogical lineage current distribution. Mitochondrial DNA has been widely used on phylogeographical studies. It has maternal inheritance, faster evolution rate than nuclear DNA and intraspecific polymorphisms. Nuclear DNA, nonetheless, could provide significant insights on male gene flow. There are few studies on phylogeographic patterns of South American crocodilians. *Caiman latirostris* is a medium sized crocodilian with a large distribution throughout South America. Phylogeography data may be used in management programs for *C. latirostris*, helping to define Evolutionary Significant Units (ESUs) for instance. Here we used mitochondrial and nuclear gene sequences to analyze the phylogeography of *Caiman latirostris* in four river basins in southeastern South America. COI mitochondrial DNA and nuclear RAG sequences were obtained from species sampled at four river basins. A total of six haplotypes were recovered for COI and one for RAG. Three distinct COI lineages were strongly correlated with river basins representing three possible ESUs. Isolation by distance was observed for the São Francisco river basin. We also observed evidence of historical demographic expansion for the populations sampled. *Caiman latirostris* ESUs harbor long-term evolutionary history and therefore they should be considered in its management and conservation programs.

Key words: *Caiman latirostris*, Phylogeography, ESUs, Conservation.

INTRODUCTION

Phylogeography investigates geographical processes influencing genealogical lineage current distribution. It takes into consideration DNA data and species natural history, as well as biogeographic events (Avise et al., 1987; Avise 2000). Phylogeography has become an important tool in conservation genetics since it helps to shed light on the definition of Evolutionary Significant Units (ESUs; Avise, 2000).

The term Evolutionary Significant Units was first proposed by Ryder (1986) as population units that show significant adaptive variation through concordance of data from different techniques. Later on Waples (1991) suggested that ESUs should be reproductively separate from other populations as well as having unique or different adaptations. Finally, Moritz (1994) defined them as populations that are reciprocally monophyletic for mtDNA and show significant divergence of allele frequencies at nuclear loci. It is important that both ecological and genetic data are used when defining ESUs (Crandall et al., 2000). If populations show strong phylogenetic divergence concordant with evidence of geographic barriers, it can be hypothesized that genetic exchangeability is prevented. This scenario results in population distinctiveness, supporting management practices that take ESUs into account (Crandall et al., 2000).

The main goal of separating populations into different ESUs should be to ensure that major historical lineages are protected and that their evolutionary potential is maintained. They are not expected to replace the concept of species in conservation initiatives, but to complement it adding information on intraspecific variability (Moritz, 1994). As highlighted by Moritz (2002) the evolutionary processes operating within the addressed taxon should be considered by conservation biologists so that adequate strategies and criteria are designed for protecting such processes and diversity within that taxon.

Since phylogeography was first mentioned by Avise et al. (1987), there has been an exponential growth in studies. This field of knowledge, however, is not yet much explored in South American countries. Until 2006 only 6.3% of the papers published on the subject in the world came from studies on South American organisms (Beheregaray, 2008). The lack of phylogeographical studies on crocodilians in South America is especially outstanding, there are only two studies on

Caiman crocodilus (Vasconcelos et al., 2006; Venegas-Anaya et al., 2008), one on *Melanosuchus niger* (Vasconcelos et al., 2008) and one on *Caiman latirostris* (Villela et al., 2008). The latter takes into consideration only part of the species distribution (coastal populations and inland populations from São Paulo state in Brazil). Therefore there is a great gap considering phylogeographical knowledge on inland populations of *C. latirostris*.

Most phylogeographical studies focus solely on uniparentally inherited markers, such as mitochondrial DNA (Beheregaray, 2008), and mtDNA does have its advantages: maternal inheritance, faster evolution rate than nuclear DNA and intraspecific polymorphisms (Avise et al., 1987). Nuclear DNA, nonetheless, could provide significant insights of past events and male correlated migratory patterns (Freeland et al., 2012).

Caiman latirostris (Daudin, 1802) is a medium sized crocodilian that reaches a maximum size of 3.5 meters but rarely outsizes 2 meters in the wild (Verdade, 1998). It has a proportionally larger snout than any other crocodilian, with a dark green coloration throughout the body with darker spots on the neck and head (Verdade & Piña, 2006). It is largely distributed throughout southeastern South America, ranging from northern Uruguay and Argentina, southern Paraguay and southwestern Bolivia (Piña et al., 2004; Verdade & Piña, 2007) to Rio Grande do Norte state in Brazil (Villela et al., 2008). This species is highly associated with lentic water bodies, such as lakes and flooded areas (Medem, 1983). More than 70% of its range is within Brazilian territory (Coutinho et al., 2012).

According to the International Union for Conservation of Nature (IUCN, 1996) *C. latirostris* has a “Least Concern” status of conservation, however the IUCN points to the need of revision and status updating. It is not included in the Brazilian list of endangered species (MMA, 2003). However, Brazilian populations are listed in CITES’ (Convention on International Trade in Endangered Species of Wild Fauna and Flora) appendix I that includes species threatened with extinction which may or may not be affected by trade (CITES, 2013). Thus, discrepant classifications among threatened species lists reflect controversy regarding the proper conservation status classification of *C. latirostris*.

Studies that consider DNA data are nowadays taken as high priority for the creation of *C. latirostris* management programs (Verdade et al., 2012). This is of extreme importance having in mind that once existing lineages are lost, they may not

be recovered unless long-term isolation occurs again, a time-scale way beyond the reaches of practical management (Moritz, 2002).

Our main objective in this study was to understand the phylogeographical history of inland populations of *Caiman latirostris* in Southeastern Brazil. First, we tested for the existence of different Evolutionary Significant Units and inferred on geographical barriers that may have influenced *C. latirostris* recent distribution; second we investigated the possibility of correlation between genetic and geographic distance (i.e. isolation by distance) and third, we investigated demographic patterns for each river basin.

MATERIALS AND METHODS

Sampling and data collection

We captured caimans at night. Young individuals were captured manually and adults, with the aid of a wire slip noose attached to a PVC pipe according to Chabreck (1963). To obtain genetic material we cut one or more scutes from the tail and stored them in 95% ethanol. We obtained tail scutes from 38 *C. latirostris* between September 2012 and June 2013 (Figure 1). We sampled four river basins: Doce, Jequitinhonha, Paraná, São Francisco. We also used sequences previously deposited in GenBank (GQ144477; GQ144478; GQ144479; GQ144480; GQ144481; GQ144482), all of them corresponding to the Paraná River Basin in Paraguay, Bolivia, and Brazil (Mato Grosso do Sul state).

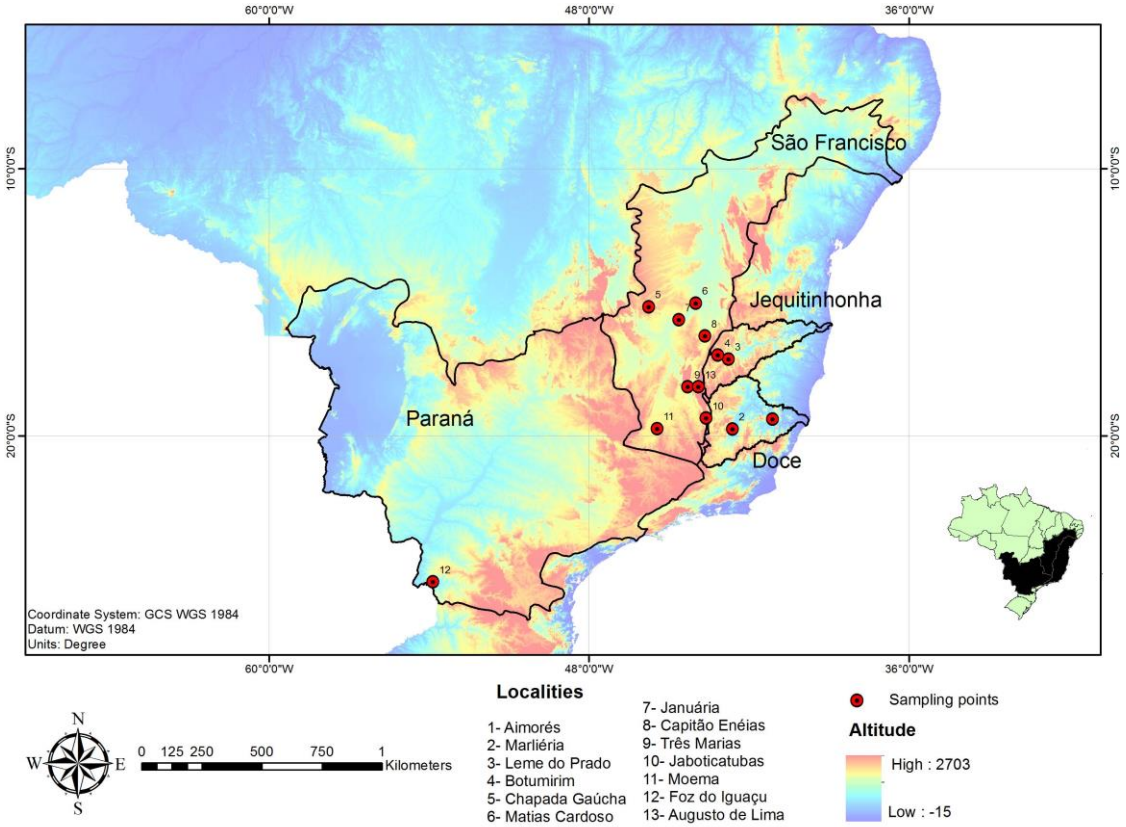


Figure 1: Localities where *Caiman latirostris* tissue samples were obtained along four hydrographic basins in southeastern South America. Black lines separate hydrographic basins.

We extracted total DNA from samples using saline extraction following a modified protocol from Aljanabi and Martinez (1997). The mitochondrial gene Cytochrome Oxidase subunit 1 (COI) was amplified with the following primers ANF1/ANR1 (M. Lyra, unpublished data) and RepCOI-F/RepCOI-R (Nagy et al.,

2012) (Table 1). PCR was carried out in a 10µl final volume following Kit Taq Phoneutria's instructions. Cycling conditions were as follows: for ANF1/ANR1, 95°C for 3min; 5 cycles of 95°C for 30s, 48°C for 30s and 60°C for 90s; 30 cycles of 95°C for 30s, 50°C for 30s and 60°C for 90s; 60°C for 3min, and subsequent storage at 10°C. And for RepCOI-F/RepCOI-R, 94°C for 3min; 40 cycles of 94°C for 40s, 48.5°C for 30s and 72°C for 60s; 72°C for 7min, and subsequent storage at 10°C. The nuclear gene RAG1 was amplified using a nested PCR with the following primers Rag1Crf.1/Rag1Crr.1 and Rag1Crf.2/Rag1Crr.2 (Hrbek et al., 2008) (Table 1). PCR was carried out in a 10µl final volume following Kit Taq Phoneutria's instructions. Cycling conditions were as following: 68°C for 60s; 35 cycles of 92°C for 15s, 52°C/55°C (Rag1Crf.1/Rag1Crr.1 and Rag1Crf.2/Rag1Crr.2 respectively) for 35s, 68°C for 90s; 68°C for 7min, and subsequent storage at 10°C.

Table 1: Primers used to amplify *Caiman latirostris* COI and RAG1 genes

Primer	Sequence	Reference
ANF1	ACHAAYCAYAAAGAYATYGG	Lyra (unpublished)
ANR1	CCRAARAATCARAADARRTGTTG	Lyra (unpublished)
RepCOI-F	TNTTMTCAACNAACCACAAAGA	Nagy et al. 2012
RepCOI-R	ACTTCTGGRTGKCCAAARAATCA	Nagy et al. 2012
Rag1Crf.1	GAGGAACTTTGCCGCATCTGTGGA	Hrbek et al., 2008
Rag1Crr.1	AGTCCTGTACATRTTRTGGTAYTG	Hrbek et al., 2008
Rag1Crf.2	tgtaaaacgacggccagtGATCTTTAAGATTGATGTGCGAGG	Hrbek et al., 2008
Rag1Crr.2	caggaaacagctatgacAAATGTATTGACTCGAATT	Hrbek et al., 2008

The lower case letters are the M13 tails of the forward and reverse primers, used for sequencing

We sequenced the genes in both directions with the Big Dye Terminator 3.1 (Applied Biosystem). Sequencing was carried out in a final volume of 10 µl, according to manufactures' instructions and read on the automatic sequencer ABI 3130 DNA Analyzer (Applied Biosystem).

We visually checked the obtained electropherograms and assembled the contig sequences on DNABaser 3.5 software. Sequences were aligned using the the ClustalW algorithm (Thompson et al., 1994) on Mega 5.2 software (Tamura et al., 2011).

We also used Mega 5.2 to calculate pairwise distances with the Kimura two parameters model (K2P) (Kimura, 1980) with 1000 bootstrap replications. A neighbor joining tree (Saitou and Nei, 1987) was constructed for graphical representation. A Maximum Likelihood (ML) (Felsenstein, 1981) tree was constructed using K2P

parameters and 1000 bootstrap replications. We used Bayesian inference on BEAST 1.8 (Drummond et al., 2012), the best nucleotide evolution model was estimated on Mega 5.2 using Akaike Information Criteria (AIC). Bayesian analysis consisted on a 50,000,000 generations run, sampling every 5,000 generations. We used a relaxed molecular clock and a constant size coalescent model starting with a randomly generated tree. Burn in was determined on Tracer 1.5 (Rambaut et al., 2013). We discarded 5,000 trees from burn in and consensus tree was taken from FigTree 1.4 (Rambaut, 2012).

To test for relationships between geographic and haplotype distributions we built a haplotype network through the Median-Joining method (Bandelt et al., 1999) on Network v. 4.6. Pairwise and geographical distances were compared with a Mantel test to search for a significant correlation among them, meaning isolation by distance (Wright, 1943), in the software R v. 3.0.2 (R Core Team, 2012) statistical package. Isolation by distance was only tested in the São Francisco river basin, since it was the only area with more than two sampling locations.

For further analysis we grouped our samples geographically according to river basins, therefore we had four major areas: São Francisco, Doce, Jequitinhonha and Paraná River Basins.

We used neutrality tests Tajima's D (Tajima, 1989) and Fu's F (Fu, 1997) on DNAsp v.5 (Librado and Rozas, 2009) to test for historical demographic expansion, negative values would indicate population expansion. We used Arlequin (Excoffier et al., 1992) to test for demographic stability in the basins using mismatch distribution of haplotypes; stable population should present multi-modal distribution whereas populations with demographic expansion should show unimodal distribution (Rogers and Harpending, 1992). The comparison of the sum of square deviations (SSD) between the observed and expected data allows to estimate significance. A significant *P* value rejects the fit of the data to the expansion model.

To check for genetic structuring we used F*st* statistics (Cockerham and Weir, 1993) and an analysis of molecular variance (AMOVA) on Arlequin 3.5 (Excoffier et al., 1992). A higher variation among basins than within basins or sampling localities as result in AMOVA would suggest genetic structuring.

RESULTS

We obtained DNA sequences of the mitochondrial gene COI from 38 *Caiman latirostris* individuals with an average length of 626bp. Six sequences from GenBank were also included, resulting in a total of 44 sequences analyzed.

RAG DNA sequences were obtained for 10 individuals from each river basin and sequences had an average length of 785bp. RAG sequences showed no polymorphism in *Caiman latirostris* (only one haplotype) and high similarity (100%) with congeneric species such as *Caiman crocodilus* (Genbank EU161708.1). Therefore, they were not included in further analyses.

The tree topologies from NJ, ML and Bayesian analyses were the same, here we show the NJ tree with three major clades with high bootstrap support and posterior probabilities. Clades were strongly correlated with river basin, supporting the presence of three separated lineages corresponding to São Francisco and Jequitinhonha, Doce and Paraná river basins respectively. K2P distances ranged from 0.2% to 2.9% and the clades were separated by distances ranging from 1.4-1.8% to 2.5-2.9% (Figure 2; Table 2).

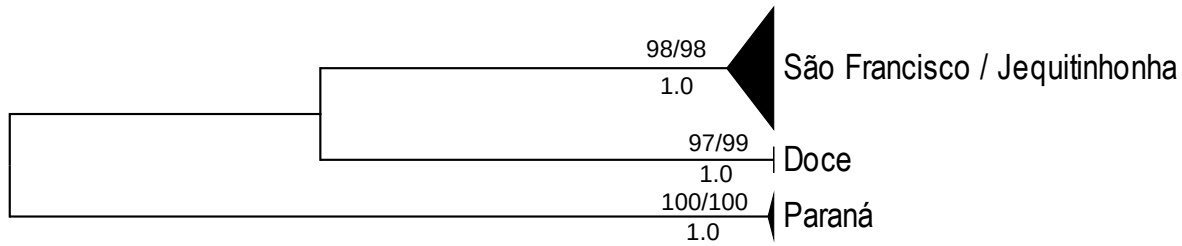


Figure 2: Neighbor joining tree based on the COI gene for *C. latirostris* showing three major clades. Numbers above the branches represent NJ and ML bootstrap values respectively and numbers under the branches indicate posterior probabilities from the Bayesian analysis.

We recovered six COI haplotypes (Figure 3). Corroborating the NJ, ML and Bayesian tree, we observed a correlation between haplotypes geographic distribution and the three clades (Figure 3; Table 2).

Table 2: Table showing *Caiman latirostris* sampling locations according to their respective Haplotype, river Basin and clades obtained from NJ, ML and Bayesian trees.

Clades	River basin	Haplotype	Localities
1	Doce	1	Aimorés Marliéria
	Jequitinhonha	2	Botumirim
2	Jequitinhonha São Francisco	3	Leme do Prado Augusto de Lima Capitão Enéias Januária Matias Cardoso Moema Três Marias
	São Franciso	4	Jaboticatubas
3	Paraná	5	Iguaçu Paraguay Bolivia
		6	Mato Grosso do Sul

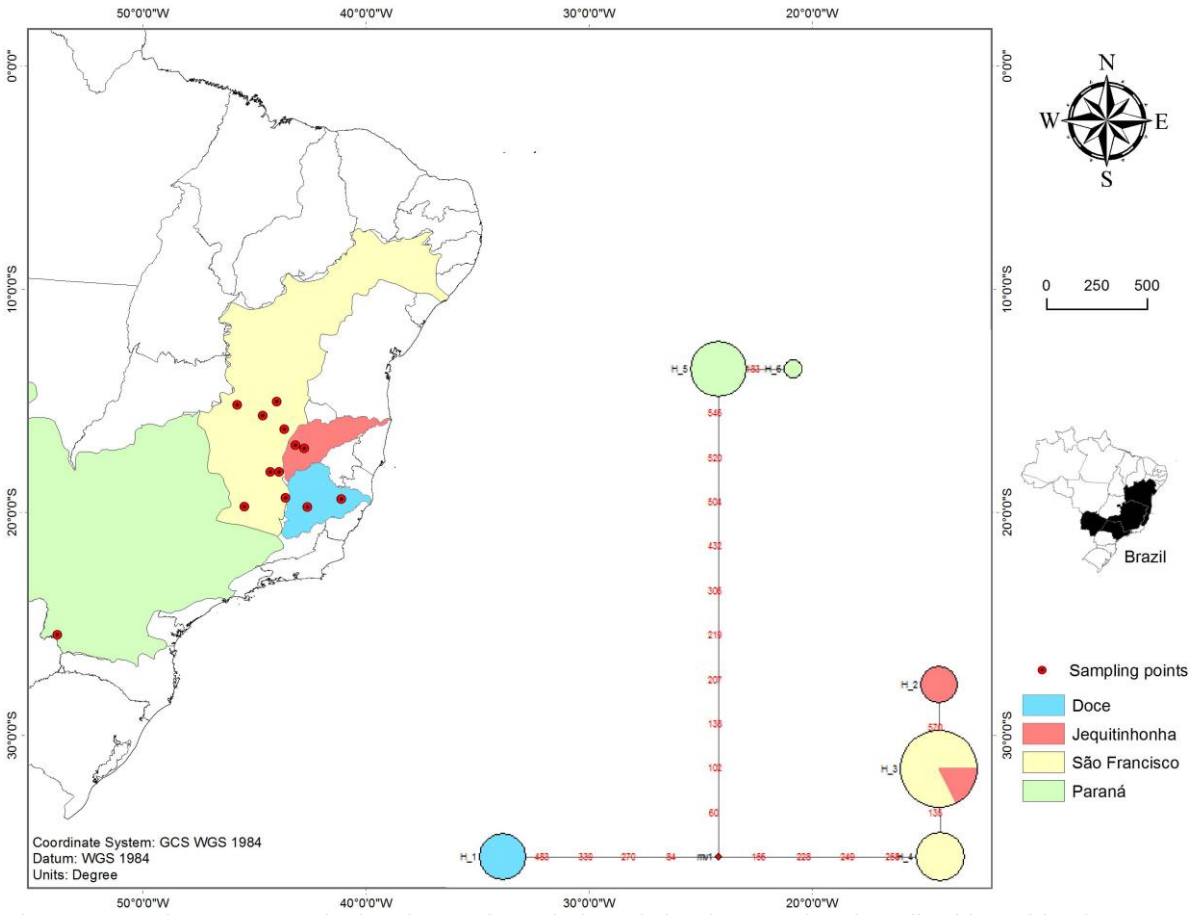
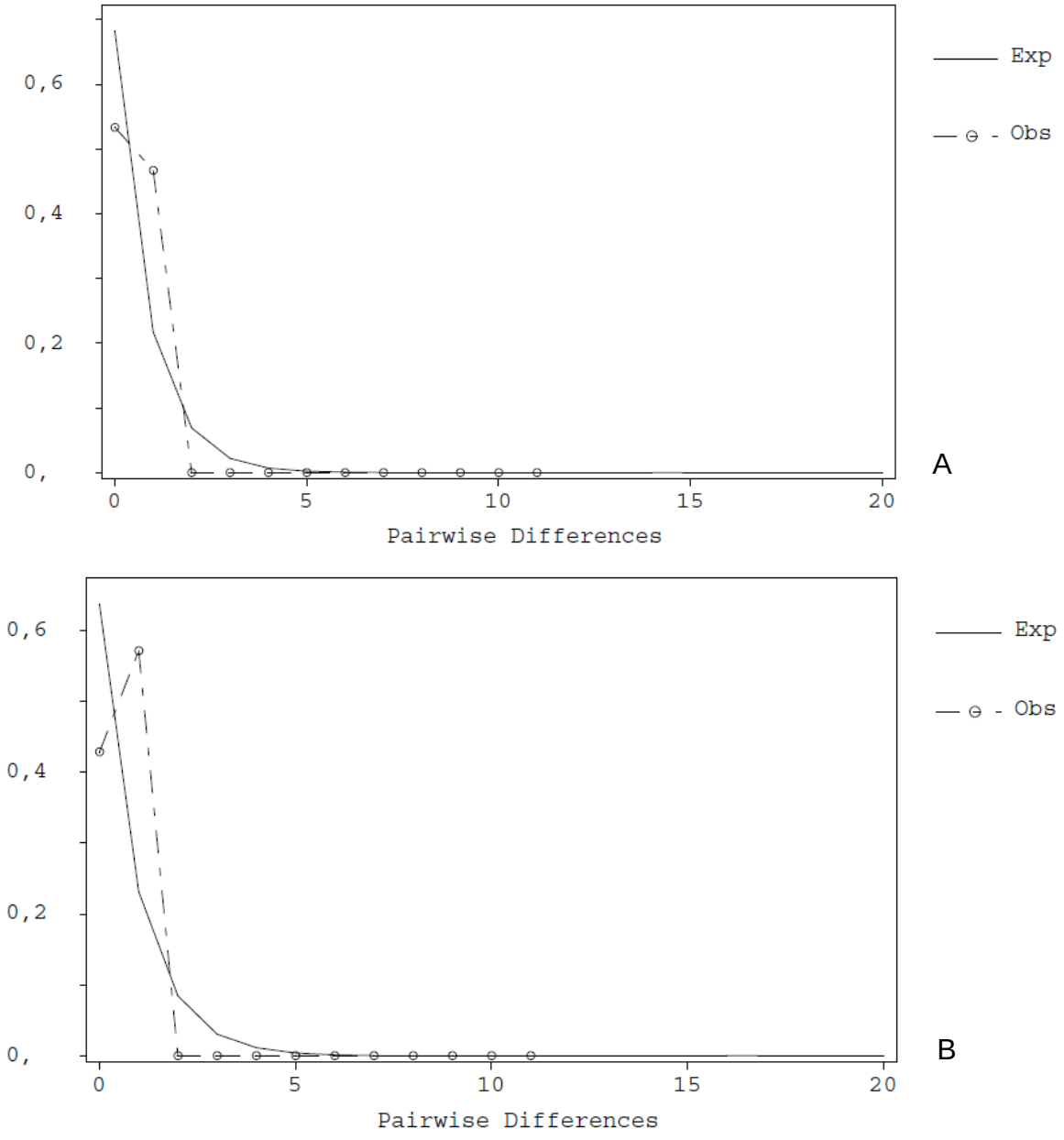


Figure 3: Haplotype network showing *Caiman latirostris* haplotypes (as described in Table 2) associated with the three observed clades in the tree topology.

The Mantel test showed that in the São Francisco River Basin there is a relationship between genetic and geographic distances, corroborating the occurrence of isolation by distance (r=0.4202, p=0.004).

Neutrality tests Tajima's D and Fu's Fs presented negative values for the Paraná basin, suggesting demographic expansion, however none of them were significant (p > 0.10) (Table 3). Plots of mismatch haplotype distribution indicated populations in all basins show signs of historical demographic expansion (Figure 4), except for Doce basin that had small mismatch distribution variance and could not have demographic parameters estimated.



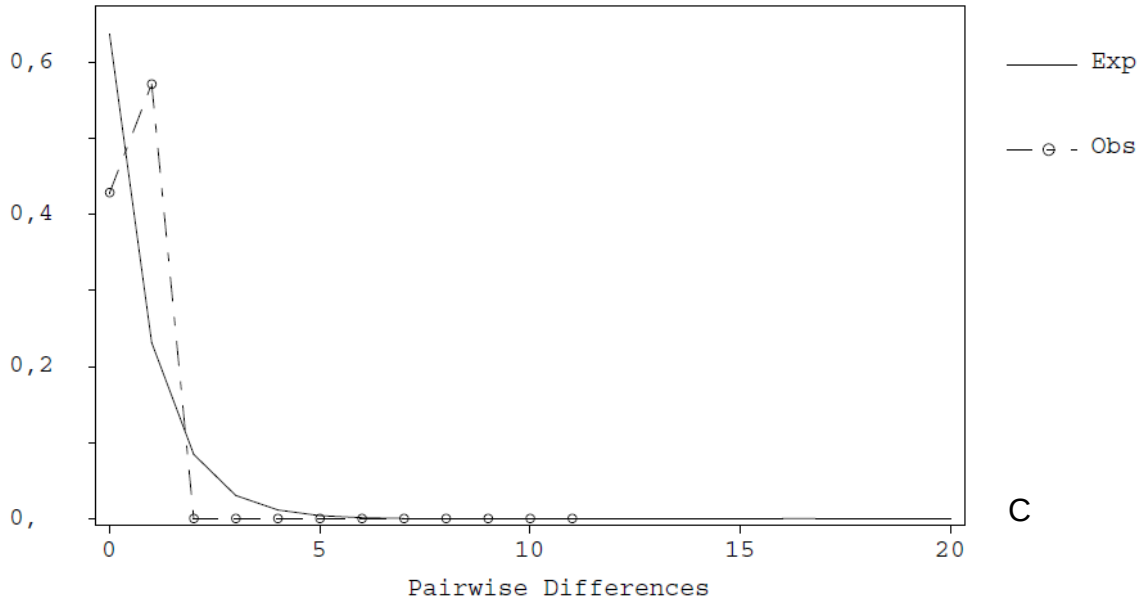


Figure 4: Mismatch graphics indicating demographic expansion for *Caiman latirostris*, based on unimodal distribution of haplotypes. A: São Francisco basin; B: Jequitinhonha basin; C: Paraná basin.

Table 3: Results of Tajima's D and Fu's Fs tests for *Caiman latirostris*. Negative values indicate population expansion, and P values indicates statistics significance (P<0.05).

Population	Tajima's D	Fu's Fs	p value
Doce	-	-	>0.10
Jequitinhonha	1.34164	1.10146	>0.10
São Francisco	1.34164	0.90565	>0.10
Paraná	-1.40085	-1.71902	>0.10

Our results showed strong genetic structuring among basins since all F*st* values were close to 1, except for Jequitinhonha and São Francisco (Table 4). All F*st* values were significant.

Table 4: F*st* values among basins demonstrating strong genetic structure for *Caiman latirostris*. Significance level = 0.05.

	Doce	Jequitinhonha	São Francisco	Paraná
Doce	0.00000	p<0.05	p<0.05	p<0.05
Jequitinhonha	0.97057	0.00000	p<0.05	p<0.05
São Francisco	0.96182	0.44332	0.00000	p<0.05
Paraná	0.98291	0.97331	0.97326	0.00000

The AMOVA also showed strong evidence for genetic structuring within river basins, since the percentage of variation among basins (94.91%) was higher than among populations within basins (4.54%) and within populations (0.55%) (Table 5).

Table 5: AMOVA results for *C. latirostris* genetic structuring, including variation percentage, fixation indices and significance level.

Source of variation	% of variation	Fixation indices	p value
Among basins	94.91	F_{CT}=0.94911	<0.0005
Among populations within basins	4.54	F_{SC}=0.89284	<0.0005
Within populations	0.55	F_{ST}=0.99455	<0.0005

DISCUSSION

Our results showed strong genetic structuring for *Caiman latirostris*. Neighbor joining tree and haplotype network results evidenced the occurrence of three major lineages along our sampled distribution of *Caiman latirostris*. One of them encompasses the São Francisco and Jequitinhonha River Basins and the other two occur within the Doce and Paraná River Basins. In Argentinian *C. latirostris*' populations, using RAPD markers, Amavet et al. (2007) found that most variations occur within populations and not among groups of populations. Our results, however, show otherwise and most variations were observed among basins, this difference could be due to the use of different molecular markers as well as a different study site

The strong genetic structuring suggests that our studied populations have been isolated from each other for an amount of time sufficient for genetic drift to occur. One single event of dispersal around 70 million years ago can account for current Alligatorinae distribution (Sill, 1968). After reaching South America in the early Tertiary, they went through a secondary radiation (Figure 5) and their occupation has in effect lasted to the present (Sill, 1968; Brochu, 2010).

The origin of mountain ranges can be a cause of lineage separation, nevertheless mountain ranges are older than the appearance of crocodilians in South America (Abreu, 1995) and therefore their geological origin could not have been responsible for the separation of lineages. They do however act as effective barriers, since *C. latirostris* do not occur in altitudes higher than 800m (Yanosky, 1992).

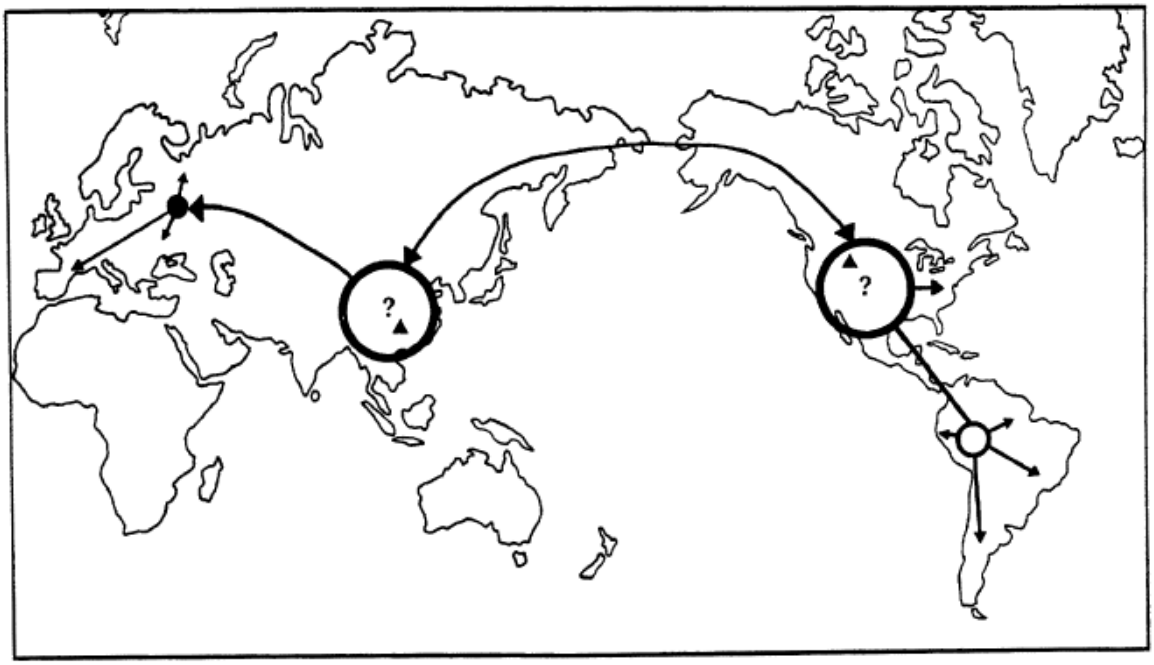


Figure 5: Alligatorinae radiation pattern showing dispersal in South America (Sill, 1968).

Villela et al. (2008) studied coastal populations of *C. latirostris* and found higher similarity between populations separated by great distances in the coast than between those geographically close to inland groups. They suggest that a large drainage area in the Brazilian coast during the Pleistocene allowed free dispersal without any major barriers. This also seems to apply to our results. Until 18 thousand years ago there was a phase of low sea levels and the formation of Pleistocene terraces (Suguio et al., 1982), therefore populations from Jequitinhonha and São Francisco should have free flow by the coast in a large drainage area. The same cannot be said for Doce populations. During this period the Doce river basin was isolated from the terraces by a saltwater lagoon (Suguio et al., 1982), this would prevent individuals from reaching others aside from those in the same basin.

Jequitinhonha and São Francisco's genetic proximity could also be explained by the capture of drainages by the Jequitinhonha basin from São Francisco (Saadi, 2002), this event however is too old to be accountable for such results.

The same theory of Pleistocene dispersal through coastal drainages can also be used to explain genetic patterns among Atlantic coastal drainage populations and Amazon basin populations of *Caiman crocodilus* and *Melanocushus niger* (Vasconcelos et al., 2006; Vasconcelos et al., 2008). Saltwater also seems to have acted as an effective barrier for *C. crocodilus* during the Pleistocene (Venegas-Anaya et al., 2008).

Although our results showed a higher percentage of differentiation among groups of populations than within them, the same was not observed for *C. crocodilus* and *M. niger*. These species presented higher differentiation within populations and within sampling localities (Vasconcelos et al., 2006; Vasconcelos et al., 2008).

Even though Tajima's D and Fu's Fs neutrality tests did not have any significant results, our *mismatch* analyses showed evidence for historical demographic expansion in all the river basins considered. Vasconcelos et al. (2006) and Vasconcelos et al. (2008) also reported demographic expansion for *C. crocodilus* and *M. niger*.

We found significant evidence of isolation by distance in the São Francisco River Basin. Isolation by distance had already been suspected for *C. latirostris* by Verdade et al. (2002) and later on confirmed by Villela et al. (2008), however both these studies used microsatellite markers. Isolation by distance is not common in crocodilians, as it has been stated by Ray et al. (2004) and Vasconcelos et al. (2008) for *Crocodylus moreletii* and *M. niger* respectively, since they have a great capacity of dispersal along bodies of water (Campos et al., 2003).

In this study we were able to clearly define three major lineages for *C. latirostris* throughout its distribution in our sampling localities. Although they do not initially present significant divergence of allele frequencies at nuclear loci, these lineages seem to have been isolated for a long period of time, do not have genetic exchangeability, have mtDNA reciprocal monophyly and therefore present compelling evidence to be considered as Evolutionary Significant Units highly associated to river basins (AMOVA, $p<0.0005$). As defined by Moritz (1994) ESUs harbor long-term evolutionary history and therefore they should be preserved due to their evolutionary potential. Future studies should focus on sampling more eastern basins and possibly cover most of the species distribution.

REFERENCES

ABREU, P. A. A. 1995. O Supergrupo Espinhaço da Serra do Espinhaço Meridional (Minas Gerais): O rifte, a bacia e o Orógeno. **Genomos**, 3(1): 1-18.

ALJANABI, S. M., & I. MARTINEZ. 1997. Universal and rapid saltextraction of high quality genomic DNA for PCR–based techniques. **NucleicAcids Res**, 25: 4692–4693.

AMAVET, P. S.; ROSSO, E. L.; MARKARIANI, R. M.; LARRIEIRA, A. 2007. Analysis of the Population Structure of Broad-Snouted Caiman *(Caiman latirostris)* in Santa Fe, Argentina, Using the RAPD technique**. Journal of Herpetology,** 41(2): 294-300.

AVISE, J. C.; ARNOLD, J.; BALL, R. M.; BERMINGHAM, E.; LAMB, T.; NEIGEL, J. E.; REEB, C. A.; SAUNDERS, N. C. 1987. Intraspecific phylogeography: the mitochondrial DNA bridge between population genetics and systematics. **Annual Review of Ecology and Systematics**, 18: 489-522.

AVISE, J. C. 2000. Phylogeography: the history and formation of specters. **Harvard University Press**, 464p.

BANDELT, H.; FORSTER, P.; ROHL, ARNE. 1999. Median-Joining networks for inferring intraspecific phylogenies. **Molecular Biology and Evolution**. 16(1): 37-48.

BEHEREGAGARAY, L. B. 2008. Twenty years of phylogeography: the state of the field and the challenges for the Southern Hemisphere. **Molecular Ecology** v-17, 3754-3774.

BROCHU, C. A. 2010. Phylogenetic relationships of *Necrosuchus ionensis* Simpson, 1937 and the early history of caimanines. **Zoological Journal of the Linnean Society,** 163: 228-256.

CAMPOS, Z.; COUTINHO, M.; MAGNUSSON, W. E. 2003. Terrestrial activity of *Caiman* in Pantanal, Brazil. **Copeia,** 3: 628-634.

CHABRECK, R. H. 1963. Methods of capturing, marking and sexing alligators. **Proceedings of the Annual Conference of the Southeast Game and Fish Commission**, 17: 47-50

CRANDAL, K. A.; BININDA-EMONDS, O. R. P.; MACE, G. M.; WAYNE, R. K. 2000. Considering evolutionary processes in conservation biology. **Tree**. 15(7): 290-295.

CITES. 2013. Appendices I, II and III. Available from <http://www.cites.org/eng/app/appendices.php>. Access on Feb 17th. 2014.

COCKERHAM, C. C.; WEIR, B. S. 1993. Estimation of gene flow from F-Statistics. **Evolution**, 47(3): 855-863.

COUTINHO, M. E.; MARIONI, B.; FARIAS, I. P.; VERDADE, L. M.; BASSETTI, L.; MENDONÇA, S. H. S. T.; VIEIRA, T. Q.; MAGNUSSON, W. E.; CAMPOS, Z. 2012. Avaliação do risco de extinção do jacaré-de-papo-amarelo *Caiman latirostris* (Daudin, 1802) no Brasil. **Biodiversidade Brasileira**. 3(1): 13-20.

DRUMMOND, A. J.; SUCHARD, M. A.; XIE, D.; RAMBAUT, A. 2012. Bayesian phylogenetics with BEAUti and the BEAST 1.7. **Molecular Biology and Evolution**, 29(8), 1969–1973.

EXCOFFIER, L., SMOUSE, P.E., AND QUATTRO, J.M. 1992. Analysis of molecular variance inferred from metric distances among DNA haplotypes: application to human mitochondrial DNA restriction data. **Genetics,** 131: 479-491.

FELSENSTEIN J. 1981. Evolutionary trees from DNA sequences: a maximum likelihood approach. **Journal of Molecular Evolution**, 17: 368-376.

FREELAND, J. R.; LIRL, H.; PETERSEN, S. D. 2012. **Molecular ecology.** 2 ed. Wiley-Blackwell, 464p.

FU, Y.-X. 1997. Statistical tests of neutrality of mutations against population growth, hitchhiking and background selection. **Genetics,** 147: 915-925.

HRBEK, T.; VASCONCELOS, W. R.; REBELO, G.; FARIAS, I. P. 2008. Phylogenetic Relationships of South American Alligatorids and the Caiman of Madeira River**. Journal of Experimental Zoology,** 309: 588–599.

IUCN. The IUCN Red List of Threatened Species. 1996. Available from <http://www.iucnredlist.org/apps/redlist/details/46585/0>. Access on Feb 17th. 2014.

KIMURA, M. 1980. A simple method for estimating evolutionary rate of base substitutions through comparative studies of nucleotide sequences. **Journal of Molecular Evolution**, 16:111-120.

LIBRADO, P.; ROZAS, J. 2009. DnaSP v5: A software for comprehensive analysis of DNA polymorphism data. **Bioinformatics,** v. 25, p.1451-1452.

MEDEM, F. 1983. **Los Crocodylia de Sur America**. Bogotá: Universidad Nacional de Colombia y Fondo "Francisco José de Caldas". 270p.

MMA (Ministério do Meio Ambiente). 2003. Instrução Normativa N° 3, de 27 de maio de 2003. Lista das Espécies da Fauna Brasileira Ameaçadas de Extinção. Diário Oficial da República Federativa do Brasil, Brasília, DF. Seção 1, 101: 88-97.

MORITZ, C. 1994. Applications of mitochondrial DNA analysis in conservation: a critical review. **Molecular Ecology,** 3: 401-411.

MORITZ, C. 2002. Strategies to protect biological diversity and the evolutionary processes that sustain it. **Systematic Biology**, 51(2): 238-254.

NAGY, Z. T.; SONET, G.; GLAW, F.; VENCES. 2012. First large-scale DNA barcoding assessment of reptiles in the biodiversity hotspot of Madagascar, based on newly designed COI primers. **PlosOne,** 7(3): 1-11.

PIÑA, C. I.; LARRIERA, A.; SIROSKI, P. 2004. Cocodrilos en la Región Litoral: especies, distribuición geográfica, modo de vida. **Miscelánia**, 12: 317-322.

R CORE TEAM. 2012. R: A language and environment for statistical computing. **R Foundation for Statistical Computing**, Vienna, Austria.

RAMBAUT, A.; SUCHARD, M. A.; XIE, D.; DRUMMOND, A. J. 2013. Tracer v1.5, Available from http://beast.bio.ed.ac.uk/Tracer.

RAMBAUT, A. 2012. FigTree 1.4. Available from http://tree.bio.ed.ac.uk/software/figtree.

RAY, D. A.; DEVER, J. A.; PLATT, S. G.; RAINWATER, T. R.; FINGER, A. G.; MCMURRY, S. T.; BATZER, M. A.; BARR, B.; STAFFORD, P. J.; MCKNIGHT, J.; DENSMORE, L. D. 2004. Low levels of nucleotide diversity in Crocodylus moreletii and evidence of hybridization with *C. acutus*. **Conservation Genetics**, 5: 449–462.

ROGERS, A.; HARPENDING, H. 1992. Population growth makes waves the in distribution of pairwise genetic differences. **Mol. Biol. Evol.,** 9:552-569.

RYDER, O. A. 1986. Species conservation and systematics: the dilema of subspecies. **Trends in Ecology and Evolution**, 1: 9-10.

SAADI, A. 2002. A geomorfologia da Serra do Espinhaço em Minas Gerais e de suas margens. **Genomos,** 3(1): 41-63

SAITOU, N.; NEI, M. 1987. The neighbour-joining method: A new method for reconstructing phylogenetic trees. **Molecular Biology and Evolution**, 4: 406-425.

SILL, W. D. 1968. The Zoogeography of the Crocodilia. **Copeia,** (1): 76-88.

SUGUIO, K.; MARTIN, L.; DOMINGUEZ, J. M. L. 1982. Evolução da planíce costeira do Rio Doce (ES) influência das flutuações do nível do mar. **Atas do IV Simpósio do Quaternário no Brasil,** 93-116.

TAJIMA, F. 1989. Statistical method for testing the neutral mutation hypothesis by DNA polymorphism. **Genetics,** 123: 585-595.

TAMURA, K.; PETERSON, D.; PETERSON, N.; STECHER, G.; NEI, M.; KUMAR, S. 2011. MEGA5: Molecular Evolutionary Genetics Analysis using Maximum Likelihood, Evolutionary Distance, and Maximum Parsimony Methods. Molecular Biology and Evolution, 28: 2731-2739.

THOMPSON, J. D.; HIGGINS, D. G.; GIBSON, T. J. 1994. CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. **Nucleic Acids Research**, 22: 4673-4680.

VASCONCELOS, W. R.; HRBEK, T.; SILVEIRA, R.; THOISY, B.; MARIONI, B.; FARIAS, I. P. 2006. Population genetic analysis of *Caiman crocodilus* (Linnaeus, 1758) from South America. **Genetics and Molecular Biology**, 29(2): 220-230.

VASCONCELOS, W. R.; HRBEK, T.; SILVEIRA, R.; THOISY, B.; MARIONI, B.; FARIAS, I. P. 2008. Phylogeographic and Conservation Genetic Analysis of the Black Caiman (*Melanosuchus niger*). **Journal Of Experimental Zoology**, 309: 600-613.

VENEGAS-ANAYA, M.; CRAWFORD, A. J.; GALVÁN, A. H. E.; SANJUR, O. I.; DENSMORE, L. D.; BERMINGHAM, E. 2008. Mitochondrial DNA Phylogeography of *Caiman crocodilus* in Mesoamerica and South America. **A Journal of Integrative Biology,** 309:614–627.

VERDADE, L. M. 1998. *Caiman latirostris.* In: ROSS, J. P. **Crocodiles. Status survey and conservation action plan**. IUCN/SSC Crocodile Specialist Group. IUCN, Gland,Switzerland, 2: 18-20.

VERDADE, L. M.; ZUCOLOTO, R. B.; COUTINHO, L. L. 2002. Microgeographic variation in *Caiman latirostris*. **Journal of Experimental Zoology**, 294: 387-396.

VERDADE, L. M.; PIÑA, C. I. 2006. *Caiman latirostris*. **Catalog of the American Society of Amphibians and Reptiles**. Salt Lake, 833: 1-21.

VERDADE, L. M.; PIÑA, C. I. 2007. Jacaré-de-papo-amarelo (*Caiman latirostris* Daudin, 1802). In: NASCIMENTO, L. B.; OLIVEIRA, M. E. **Herpetologia no Brasil II**. Belo Horizonte: Sociedade Brasileira de Herpetologia, p. 295-307.

VERDADE, L. M.; LARRIERA, A.; PIÑA, C. I. 2012. Broad-snouted Caiman *Caiman latirostris*. **CSG Action Plans**. Disponível em:

<http://www.iucncsg.org/365_docs/attachments/protarea/04_C-c7ff4560.pdf>. Access on May 3rd. 2012.

VILLELA, P. M. S.; COUTINHO, L. L.; PIÑA, C. I.; VERDADE, L. M. 2008. Macrogeographic variation in Broad-Snouted Caiman (*Caiman latirostris*). **Journal of Experimental Zoology**. 309A(10): 628-636.

WAPLES, R. S. 1991. Pacific Salmon, *Oncorhynchus* spp., and the Definition of "Species" Under the Endangered Species Act. **Marine Fisheries Review**. 53: 11-22.

YANOSKY, A. A. 1992. Una población de yacarés overos (*Caiman latirostris*) a 800 msnm. IN: **II Congreso Argentino de Herpetología.** La Plata, Argentina. p. 185-189.