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HIBRIDAÇÃO E INTROGRESSÃO EM ZONA DE CONTATO ENTRE ALOUATTA GUARIBA CLAMITANS E ALOUATTA CARAYA (PRIMATES) NO SUL DO BRASIL ESTUDADAS COM DADOS GENÔMICOS

Porto Alegre
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Dissertação apresentada como requisito para a obtenção do grau de Mestre pelo Programa de Pós-Graduação em Zoologia da Faculdade de Biociências da Pontifícia Universidade Católica do Rio Grande do Sul.

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Co-orientador: Dr. Italo Mourthé

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SUMÁRIO

Aos bugios, eu dedico esta dissertação!

“O que faz andar a estrada?
É o sonho. Enquanto a gente sonhar
A estrada permanecerá viva.
É para isso que servem os caminhos,
Para nos fazerem parentes do futuro”

Mia Couto

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Resumo

Zonas de contato,com presença de bandos mistos,entre *Alouatta caraya* e *Alouatta guariba clamitans*, tem sido recentemente documentada e a hibridação foi inferida pela presença de indivíduos com padrões morfológicos mistos. Neste estudo nós sequenciamos milhares de regiões ultra conservadas em amostras não-invasivas de fezes dentro de uma zona hibrida e de populações adjacentes das duas espécies e caracterizamos 3302 sítios polimórficos.Nós encontramos híbridos F1 limitados a zona de contato, derivados das duas combinações de parentesco, mas também encontramos evidência de híbridos na zona de *A. g. clamitans* originados de retrocruzamentos por várias gerações de híbridos comindivíduos provavelmente puros de *A. g. clamitans*. Não existe evidência de nenhum introgressão maternal (mtDNA), e somente duas fêmeas hibridas F1, então fêmeas hibridas F1 parecem ser inviáveis ou inférteis. Por outro lado, encontramos introgressão bidirecional do cromossomo Y a pelo menos dezenas de quilômetros além da zona de contato em ambos os lados,em indivíduos sem outro sinal de introgressão, sinalizando uma introgressão antiga mediada pelos machos.

Palavras-chave: Ultraconserved Elements, hibridação, DNA fecal, bugio, sequenciamento de alto desempenho

Abstract

Contact zones between *Alouatta caraya* and *A.guariba clamitans*with mixed groups has been recently documented and hybridization was inferredby the presence of individuals with mixed morphological patterns. Here we sequenced thousands of ultraconserved elements in non-invasive fecal samples within a hybrid zone and from adjacent populations from both species and characterize 3302 polymorphic sites. We found several F1 hybrids in a narrow contact zones, derived from both combinations of parents, but also found evidence of hybridsin the *A. g. clamitans* zone that likely originated from serial backcrosses of hybrid males with purebred *A. g. clamitans* females. There is no evidence of any maternal (mtDNA) introgression, and only two female F1 hybrids were found, therefore female F1 hybrids seem inviable or infertile. On the other hand, we found bidirectional introgression of the Y-chromosomes at least tens of kilometers away from both sides of the contact zone, in otherwise apparently purebred individuals, indicating a not recent introgression mediated by males.

Keywords: Ultraconserved Elements, Hybridization, Fecal DNA, Howler Monkey, high throughput sequencing

Apresentação

Regiões de simpatria entre *Alouatta* são descritas ao longo de toda distribuição deste gênero. As zonas de contato registradas até então foram entre *A. pigra* e *A. palliata* no México (Cortés-Ortiz *et al*. 2003; Baumgarten & Williamson 2007), *A. palliata*e *A. seniculus* na Colômbia (Defler 2004) e no Brasil entre *A. seniculus* e *A. belzebul* (Hirsch *et al*. 1991; Pinto & Setz 2000),*A. seniculus* e *A. caraya*(Wallace *et al*. 2000; Iwanaga & Ferrari 2002), *A. belzebul* e *A. caraya*(Chame & Olmos 1997) e *A. caraya*e *A. guariba clamitans* (Hirsch *et al*. 1991; Di Bitetti *et al*. 1994; Gregorin 2006; Aguiar*et al*. 2007, 2008; Passos *et al*. 2007; Agostini*et al*. 2008). No entanto, há confirmação de hibridação baseada em dados genéticos apenas entre *A. palliata* e *A. pigra* simpátricos na região de Tabasco, México (Cortés-Ortiz *et al*. 2007).

O primeiro registro da presença de indivíduos *A.caraya* e *A. g. clamitans* no Rio Grande do Sul foi feito pelo naturalista francês Arsène Isabelle em 1833:

> " ... Encontram-se nesta localidade muitos macacos do gênero alovatte, chamados bugios pelos brasileiros. Matamos um velho macho, muito barbudo, quase vermelho; a fêmea que ferimos tinha um pêlo esbranquiçado como o do uivador negro; quanto ao jovem macho de menos de dois anos, variava de cor desde o branco até o marrom vermelho. Deparamos com eles freqüentemente, nos grandes matos, até Porto Alegre, pelos 30° de *latitude*, e posso afirmar positivamente que existem ao sul de *Caçapava*, sob o paralelo 31, contrário à opinião de um célebre viajante, que julga que o limite geográfico dos macacos é a 27° graus, na parte austral do novo continente..." (Isabelle, 1983, p28)

Behling *et al.,*(2005) inferem que a região de São Francisco de Assis, coberta por campos nos últimos 22000 anos, começou a ser sobreposta há 5000 anos, por matas de galeria composta por flora de áreas típicas do habitat de *A. caraya*e de *A. g. clamitans.* Sendo este fenômeno proeminente nos últimos 1500 anos. Segundo Bicca-Marques *et al*. (2008), esta zona de contato é, provavelmente, recente se consideradas as histórias evolutivas destas espécies. A expansão das matas de galeria sobre os campos e o processo de dispersão destes primatas possivelmente

proporcionou o contato entre estes e outros táxons na região (Behling *et al*., 2005; Bicca-Marques *et al*., 2008, Trigo *et al*., 2008).

Esta dissertação teve como objetivo caracterizar esta zona híbrida e o nível de introgressão entre estas espécies e também de populações adjacentes potencialmente puras, através do sequenciamento de milhares de locos de amostras fecais, avaliando as possíveis causas para estes fenômenos. Esta dissertação foi organizada em formato de um manuscrito em preparação a ser submetido ao periódico Molecular Ecology.

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--- Manuscrito a ser submetido ao periódico Molecular Ecology ---

Asymmetric and male-biased introgression in a hybrid zone between howler monkeysshown by genomic evidences from non-invasive samples

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Keywords: Ultraconserved Elements, Hybridization, Fecal DNA, Howler Monkey, high throughput sequencing

Abstract

Contact zones between *Alouatta caraya* and *A. guariba clamitans* with mixed groups has been recently documented and hybridization was inferred by the presence of individuals with mixed morphological patterns. Here we sequenced thousands of ultraconserved elements in non-invasive fecal samples within a hybrid zone and from adjacent populations of both species. We characterized 3302 polymorphic sites and found several F1 hybrids in a narrow contact zone, derived from both combinations of parents. We also found evidence of hybrids in the *A. g. clamitans* zone that likely originated from serial backcrosses of hybrid males with purebred *A. g. clamitans* females. There is no evidence of any maternal (*mtDNA*) introgression, and only two female F1 hybrids were found, suggesting female F1 hybrids may be inviable or infertile. On the other hand, we found bidirectional introgression of the Y-chromosomes at least tens of kilometers away from both sides of the contact zone, in otherwise apparently purebred individuals, indicating a not recent introgression mediated by males.

Introduction

Natural hybridization among animals is more common than previously expected (e.g. Arnold 2004, Mallet 2005, Dowling and Secor 1997, Kelaita *et al*. 2013), occurring when different taxa crosses producing at least some viable F1 progeny with some level of fertility (Arnold 1997). Different mechanisms, such as evolutionary, demographic, behavioral, and ecological processes may work together to sustain the hybridization process (Cortes Ortiz *et al.* 2015).

Diverging populations can hybridize even after considerable periods of time and traces of introgression, by secondary contact, can be found in the genome of several taxa (Rosenzweig *et al*. 2016). If hybrids are viable, they can backcross with their parental species or crossbreed with other hybrid individual, producing offspring with different levels of genetic admixture (Cortés-Ortiz *et al*. 2015).

Usually, studies addressing species hybridization are mostly based on morphological features of the target taxa, depending on the researcher's ability to recognize signs of hybridization on the phenotype (Ackermann *et al*. 2006). Hybrid individuals usually present conspicuous characteristics in mammals, such as color variation, changes in body size, and tooth numbers (Mallet 2007). For example, there are well-documented variations in color patterns among primates (Ackermann 2010) and small felids (Trigo *et al*. 2008).

The first reports of hybridization between primates in the wild were described in *Macaca*, based on phenotypic differences between the potential hybrids and pure individuals (Bernsteil, 1966). Since that, several authors described primate putative hybrids based on mixed patterns on morphology and behavior. The extensive widespread of molecular techniques in the last decades however allowed a steady increase in the number of studies confirming the hybridization among several primate taxa (Merker *et al*. 2009; Cortés-Ortiz *et al*. 2007; Wyner *et al*.2002; da Silva *et al*. 1992).

Alouatta is the most widespread genus occurring from southern Mexico to southern Brazil, including at least 9-11 species that occurs mostly in allopatry (Gregorin 2006; Cortés-Ortiz *et al*. 2015a). There are 11 contact zones reported along the *Alouatta* distribution (Cortés-Ortiz *et al*. 2015). As usual, most studies describing putative howler hybrids were based on the intermediate or mosaic phenotype, or mixed behavior of a few individuals (Cortés-Ortiz *et al*. 2003, 2007; Gregorin 2006; Aguiar *et al*. 2007; Agostini *et al*. 2008; Bicca-Marques *et al*. 2008; Silva 2010). The first molecular evidence confirming the hybridization in howlers came from the Tabasco department, Mexico between *A. palliata* and *A. pigra* (Cortés-Ortiz *et al*. 2007).

It was suggested the presence of unidirectional hybridization in this pair, in which the crossing between males of *A. palliata* and females of *A. pigra* only produced F1 fertile females. It reinforces the prediction of Haldane's rule, which establishes that it is more likely for the heterogametic sex (males in mammals) to be inviable or sterile (Haldane 1922). Even so, the genetic variability at the uni- and bi-parentally inherited loci found among hybrids showed that backcrossing was occurring and the production of fertile multigenerational backcrossed males was possible (Cortés-Ortiz *et al*. 2007). Additionally, it is hypothesized that beneficial adaptations may be achieved by the animals once they may inherit physical or behavioral traits that confer a fitness advantage to the hybridized individuals (Kelaita and Cortés-Ortiz 2013). For example, hybrid males may inherit morphological features (as larger body size) from one of the parental species that aid them in competing with males from the other parental specie. Therefore, despite the potential genetic, demographic, or social obstacles for producing first generation hybrids, viable and fertile F1 individuals may have a fitness advantage and continue to backcross with either purebred or backcrossed individuals, resulting in the observed large number of multigenerational backcrossed individuals (Kelaita & Cortés-Ortiz 2013; Cortés Ortiz *et al*. 2015). The cross between different howlers may be facilitated by fragmentation, an exogenous mechanism of influence (Dias *et al*. 2013).

Two species of howler monkeys occur in the southern Brazil, the red howler monkey, *Alouatta guariba clamitans*, and the black howler monkey, *Alouatta caraya*. They are globally classified as Least Concern in the IUCN Red List (The World Conservation Union), with a trend for population decreasing (Fernandez-Duque *et al*. 2008; Mendes *et al*. 2008). The first signs of putative hybridization between these southern howlers was based on museum specimens with mosaic pelage collected in ecotone habitats in the Parana State, southern Brazil in 1940s (Lorini and Persson 1990; Gregorin 2006). Mixed groups composed by *A. caraya* and *A. g. clamitans* was continually reported (Isabelle 1983; Aguiar *et al*. 2007, 2008, 2014). So far, there are six areas of sympatry described between *A. caraya*and *A. g. clamitans* (Agostini *et al*. 2008; Di Bitetti 2005; Bicca-Marques *et al*. 2008; Aguiar *et al*. 2007; 2014; Gregorin 2006; Holzmann *et al*. 2015).

Alouatta g. clamitans and *A. caraya* occur in the Atlantic Forest and in the *Pampa*, respectively, in the Rio Grande do Sul (Bicca-Marques *et al*. 2008) (Fig. 1). The contact zone between these species is also located within the ecotone between these two biomes, both species have similar trophic niches and they are quite tolerant to habitat disturbance (Zunino *et al*. 2007; Bicca-Marques *et al*. 2008; Agostini *et al*. 2010). Based on population surveys, Bicca-Marques *et al*. (2008) suggested that the area of contact between *A. caraya*and *A. g. clamitans*in São Francisco de Assis (RS) may extended over a 20-km strip based on mixed groups of howlers found in this area. Individuals with mixed pelage, presenting color patterns as putative hybrids (Aguiar *et al*. 2007,2008) were found in this region, inferring the hybridization based on morphological patterns.

Many studies have used non-invasive DNA samples (e.g. feces) and traditional microsatellite markers to determine pure and admixed individuals. The use of non-invasive methods to isolate DNA is ideal for genetic studies of endangered natural populations (Perry *et al*. 2010). Fecal samples can be good sources of genetic information; especially in herbivorous

however, the concentrations of endogenous DNA in fecal samples are in low quantity (e.g. 1.8%, Perry *et al*. 2010) and quality. Due to these limitations, genetic analyses of DNA from non-invasive samples using traditional techniques have largely been restricted to the mitochondrial DNA sequencing and genotyping small numbers of microsatellite loci (e.g. Fagundes *et al*. 2008; Chaves *et al.*2006).

We have recently provided the only genetic evidence of hybrids between these species, based on mitochondrial and Y-chromosome markers, and a few microsatellite loci (Mourthe *et al*., in prep.). However, with these markers we could only determine the evidence of putative F1 hybrids, as their uniparental non-recombining nature do not allow further characterization of the process. There are several open questions here, such as: the viability and fertility of the hybrids; if there was introgression beyond the narrow contact zone and possible asymmetries in this process, both between sexes and between parental species; for how long hybridization have been occurring; the possible causes (natural or anthropogenic) of these contact zone. These questions could only be answered by incorporating large number of markers (e.g. thousands) over the genome.

One of the more innovative and powerful of these techniques uses Ultraconserved Elements (UCEs) that are highly conserved regions of the genome shared among evolutionary distance taxa (e.g., birds share many UCEs with humans). UCEs were shown useful for reconstructing the evolutionary history and population-level relationships of many organisms, enable target enrichment and sequencing of thousands of orthologous loci across species separated by millions years of evolution (Faircloth *et al*. 2012). The high increase in the number of molecular markers used in such next generation sequencing technique enable a higher precision in the analyses than using traditional markers in studies of population genetics and hybridization (Ekblom & Galindo 2011; McCormack *et al*. 2013; Keller *et al*. 2013; Catchen *et al*. 2014).

In this study, we survey a hybrid zone of *Alouatta caraya* and *A. g. clamitans* using UCEs to sequence thousands of loci in fecal samples to characterize the patterns of genetic introgression between these primates in the southernmost part of their distributions in Brazil.

Material and Methods

Study area

The surveys were done in forest fragments in the state of Rio Grande do Sul, southern Brazil (Fig. 1). First,in a contact zone previously described (by putative hybrids – Bicca-Marques et al. 2008) in São Francisco de Assis (29.5549° S; 55.1257° W) and São Vicente do Sul (29.6920° S; 54.6768° W) which is ecotone region between Atlantic Forest and Pampa biome (Figs. 1 and 2).We also collected fecal samples outside this zone, where only phenotypical pure individuals were found, that were then considered at first as representing the parental populationsin Alegrete (~50 km from contact zone,29.7906° S; 55.7954° W) and Santa Maria (~85km from the contact zone,29.6873° S; 53.8154° W), respectively.

Sampling collection

We collected 89 fecal samples of *A. caraya* and *A. g. clamitans* within the Contact Zone between these species, from 2010 to 2014. On the AC Zone we collected of *A. caraya* (N=10) and on AG Zone *A. g. clamitans* (N=9).

During the surveys, we searched for howler monkey groups and followed them until they defecate. Individual fecal samples were collected using disposable gloves and storedin ethanol 70% in the field. Once in the laboratory, samples were kept at -20°C. Only fresh fecal samples were collected for this study. Groups sighted in the same day and wereat least 300m of distance were considereddifferent groups.

Traditional molecular methods

Fecal DNA extraction was performed using the QIAamp DNA Stool Mini Kit® (Qiagen) following the manufacturer's instructions. To avoid contamination of exogenous DNA, extractionswere performedin a separate room, specific for handling non-invasive and/or hard DNA samples. We included a negative control(reagents only) in each batch of extraction to monitor for cross-contamination during the procedure. The presence and quality of the genomic DNA extracted was verified by running all DNA samples in 1% electrophoresis gel stained with GelRed 10x (Biothium).

A 563-bp fragment of the mitochondrial control region DNA was amplified by Polymerase Chain Reaction (PCR) using the primers RCBugioR and HowRA1 (Ascunce *et al*. 2003). PCR products were then purified and sequenced.Controlregion is known to clearly distinct the two species, and presents, besides several diagnostic mutations, a 58 bp deletion between AC and AG, allowing the precise species identification of the origin of the mtDNA.

Additionally, a300-bp segment of the *SRY* gene of the Y chromosomewere amplified for males using the pair of primers *SRY*F2 (5' AAAGTAACAACGAATTTGGTAGAA3') and posteriorly sequenced. We have previously determined the presence of three fixed diagnostic mutations between the *A. caraya* and *A. guariba*in this fragment(Mourthe *et al*., in prep.) so it was used to infer the origin of the Y chromosome in samples from both species.Also, the presence/absence of the *SRY* amplification was used as a sex determination tool for the fecal samples, this was done at least twice. Finally, three microsatellite locithat are known to presentspecies-specific alleles were genotyped for the samples, although only a subset of samples could be efficiently genotyped due to lower amount of genomic DNA (See Results). All details regarding datageneration of SRY and microsatellite results mentioned above can be found in Mourthé *et al*. (in prep).

Ultraconserved Elements capture from fecal DNA

Fecal DNA usually tends to containmore exogenous than endogenous DNA (REF).For example, Perry et al. (2010) estimated only 1.8% of endogenous DNA infecal samples of chimpanzees. To determine the endogenous DNA in our samples we amplifiedthe autosomal microsatellite locus AC17by PCR, which is known to be monomorphic in howler monkeys(Oklander *et al*. 2007; Mourthe*et al*. in prep.). We estimated the amount of endogenous DNA based on the intensity of PCR band in a 2% agarose gel, which were compared to PCR productsobtained through amplification of the same locus fromDNA extracted fromblood samples. We then quantified the amount of DNA by using Low Mass Ladder (Invitrogen)…. Acho que tem q explicar como foi feita essa quantificação :-/

The ultraconserved elements capture was performed by using a set of 5,472 comercial probes targeting 5,060 UCE fromtetrapods (MYcroarray, Inc.). Details on probe design and characteristics can be accessed at http://ultraconserved.org (last accessed on March 15, 2016). For genomic libraries construction,100 µl of each DNA extract (with concentration ranging from 0.0009 to 9ng/µl)was mechanically fragmented by sonication with Biorruptor (Diagenode).We applied 3-8 cycles on high speed with on/off intervals of 30s to shear the DNA in fragments of 200-600 bp. After sonication, 5 µl of each sample was run on a 1.5% agarose gel along with a Low Mass Ladder as a fragment size guide,to verify if the smear DNA fits the required DNA size range. When necessary, a second round of sonication was performed (one or two more cycles usually) until DNA fragments reach the desired bp-lenght.

We initially prepared dual-index Illumina genomic libraries based onthe protocol outlined by Meyer & Kirchner (2010) with some modifications (tem que ver com o Sandro se mencionamos que foi Seramag beads ao invest de AMPure).After the Fill-in step we employed an enzyme inactivation step instead of the bead clean up according to Kircher et al. (2011). Libraries were then amplified twice by PCR according to protocol described by Kircher et al.

(2011). Second round of amplification was perfomed with Kapa ReadyMix (REF). PCR products were pufiried using QIAquick (Qiagen) and eluted in 10ul. Each library was quantified by Nanodrop before pooling.We followed the libraryenrichment procedures for the MYcroarray MYBaitskit and custom sequence tags unique to each sample (Faircloth and Glenn 2012), following a limited 14-cycle PCR to enrichthe indexed libraries. We defined eleven pools combining eight dual-indexed individual libraries each by grouping samples with similar concentration. Pools were then subjected to enrichment with MYbaits kitfollowing User's Manual v3.0 (MYcroarray). Post-capture PCR reactions were purified using QIAquick kit (Qiagen) and eluted in 25ul. Enriched library pools were quantified by qPCR using the Kapa qPCR Mix(Kapa Biosystems, Inc.) and then mixed in homogeneous amounts in a single pool for sequencing.To test the efficiency of the library construction and capture expertiment, a first set of eight individuals was sequenced, and was diluted to represent about 5% of the total Illumina HiSeq2000 lane.Based on the obtained results, anotherpoolcontaining 81 indiduals was sequenced in asinglelane of a 100-bp single-end Illumina HiSeq 2000 run (Sickkids genome lab, Canada),totaling 89 howley monkey individuals sequenced.

Bioinformaticsanalyses

Sequence reads were received separated by each barcode sequence. We filtered reads for adapter contamination, low-quality ends, and ambiguous bases using an automated pipeline (https://github.com/faircloth-lab/illumiprocessor) that incorporates Scythe (https://github.com/vsbuffalo/scythe) and Sickle (https://github.com/najoshi/sickle). Subsequently, we produced consensus contigs *de novo* for all reads generated using VelvetOptimizer (S Gladman; https://bioinformatics.net.au/software.shtml) and VELVET (Zerbino and Birney 2008). Consensus contigs were aligned to the UCE probes using LASTZ (Harris 2007). Any contig that did not match a probe were removed. We used BWA (Li and

Durbin 2009) to generate an index of consensus UCE contigs and mapped all reads to the UCEs probes again, for test. After mapping, we called SNPs and indels for each individual and exported BAM pileups using SAMtools (Li *et al*. 2009). SAMtools were used to make individual-specific consensus sequences for each SNP and filtered low-quality bases (>Q30). SNPs were converted to a flat table format using a custom Perl script which removed indels, required sites to have QUAL > 20,and converted FASTQ file to FASTA format, to use PLINK. Then, for the final dataset we filtered UCEs with >0.25 missing data and retaining a single SNP per loci, the one with less missing data.

Population genetics

We used ADMIXTURE 1.23 (Alexander *et al*. 2009) toidentify the genetic composition of the parental species (*A. caraya*and*A. g. clamitans*) and therefore identify putative hybrids, setting K = 2 genetic clusters. We also tested the number of clusters from K=1 to 10 using the approach described in the manual. We similarly also used STRUCTURE 2.3.4 (Pritchard *et al*. 2000) asan independent corroboration of the genetic structure of the data set. STRUCTURE was run with a burn-in of 250K steps followed by another 250K of MCMC steps, assuming an admixture model and correlated allele frequencies. We run this analysis without any prior information of taxon identify, and tested the number of cluster from K=2 to K=10, with 10 replications. Results were averaged across replicates using R in PopHelper package (Francis 2016). The estimated membership coefficients Q for each individual in each cluster was calculated, in order to assign the individual to one or, if admixed, to both clusters.

We used R scripts available from the Prediction of Populations Structure (POPS) website to plot the results of Structure cluster membership on the geographic distribution of the samples.A principal coordinate analysis (PCoA) between individuals was performed in R (cmdscale function; R Core Team 2014) using Euclidean distances on the SNP dataset between individuals.

GeneClass2?

Results

In total, we studied 89 samples: 10 from *A. caraya* pure zone(AC), 9 from *A. g. clamitans* pure zone (AG), and 70 from the hybrid zone, that represent 88 different individuals (one sample was replicated). These samples represent 27 howler groups (Fig. 2) distributed along a total of 199 km (see Table S1 for geographic coordinates). The quantification of the endogenous DNA on the fecal extracts based on a microsatellite amplification varied from 0.001 – 0.1 ng/µl. This confirmed the very smallamount of endogenous DNA on these monkeys'fecal samples.

After the initial processing with Illumiprocessor we input 87 million trimmed reads into the assembly process (Table 1 and S2). The assembly results in about 5 million reads into contigs with average length of 546 bp. After applying filters as explained in the Material and Methods, we recovered a total of 3499 UCE loci with at least one SNP in all samples. However, for the final data set we also filter UCEs >0.25 missing data and retained a single SNP per UCE loci, obtained a total of 3302 UCE loci and the same number of polymorphic sites. The level of missing loci per individual ranged from 2% to 96% (Table S3). Giventhe main objective was to identity hybrids, and for that even a few loci may be informative, we decided to use all individuals in most analyses.

The result of the ADMIXTURE analyses from K=1 to K=10 (Fig. S1) corroborates the existence of two main genetic groups that could be assigned to *A. caraya* and *A. g. clamitans* (Fig. 3). These analyses show the presence of purebred individuals of both species and corroborates the presence of several individuals that are likely F1 hybrids and others with variable proportions of the genetic components of the two species. We will mostly use the ADMIXTURE result from now on, but the STRUCTURE result with K=2 is very similar (Fig. S2), the main difference being some individuals considered purebreds with the former. Using the

above results to define purebred individuals, we found 133 SNPs that are fixed between the species and 131 fixed SNPs if we considered only purebreds outside the contact zone.

All 10 individuals from the *A. caraya* zone and in the contact zone up to group B12 are purebred *A. caraya* (three has <4% of *A. g. clamitans* component and one <8%). On the other hand, only two of nine individuals of *A. g. clamitans* zone are purebred, the others have between 12% and 40% of *A. caraya* component. The groups from B13 to B22 in the contact zone present individuals with very different ancestries. For example, three of four individuals of B22, that is the closest to the *A. g. clamitans* zone, are purebred *A. g. clamitans* and one present <10% of the other component and the four individuals of B21 (the southernmost group) that are purebred *A. caraya*. All the other eight groups present at least some hybrid (or all) individuals, although group sample sizes may be as small as a single individual.The nine individuals whose proportion of both components are between 0.41 and 0.59 are here considered F1 hybrids (7 males and 2 females). Notable is group B17, with purebreds of both species and a F1 hybrid and B15, with six different samples (one was a replicate), four purebred *A.caraya* and two F1 hybrids. The geographic distribution of the proportion of the genetic components (Fig. 4) shows a clear west-east gradient from the *A. caraya*to *A. g. clamitans*, but note the asymmetric distribution, with purebred A. *caraya*occurring into the contact zone.

When the ADMIXTURE plot was ordered not by the geography but by the proportion of the *A. caraya* component (Fig. S3), we again see the pattern of admixture is asymmetric, with an abrupt decreasefrom pure or almost pure *A. caraya* to highly admixed ones while the decrease of the *A. g. clamitans* component is smoother.

The PCoA results (Fig. 5 and S4) corroborates the resultsabove. The purebred individuals are located on opposite sides of PC1 axis, the putative F1 hybrids in the intermediate area while the individuals with the proportion of the *A. g. clamitans* component between 0.6 and 0.9

(probably backcrossed hybrids, with *A. g. clamitans*) are between the F1 hybrids and purebred *A. g. clamitans*.

Finally, we compared our bi-parental NGS results with mtDNA maternal and Y-chromosome paternal uniparental markers (Fig. 3), sex determination, and evidence of three diagnostic microsatellite loci (Mourthe*et al*. in prep.). First, the mtDNA present a complete concordance with both geography and whole genomic composition. That is, all individuals from both pure zones present mtDNA from the correspondent species and all individuals that are not F1 hybrids have the mtDNA in accordance with their major genetic component, suggesting no mtDNA introgression. We found 37 males from the 88 individuals, given a M:F ratio of 0.73, while a previous visual survey on a nearby contact zone found 0.87 ratio, suggesting we may have at most a few (~4) undetected males on our results.Different from mtDNA, we found widespread bidirectional Y-chromosome introgression, on both pure zones as well as on individuals that were otherwise considered purebreds, as the case for seven *A. caraya* males, suggestion ancient bidirectional introgression exclusively mediated by male. Finally, of the eight candidate F1 hybrids based on the microsatellite data studied here only one was not corroborate with our UCE results.

The identification of the maternal parental of all F1 hybrids as well as the male parental of all male hybrids, together with the NGS results, allow us to better characterize the outcomes of crosses between *A. caraya* and *A. g. clamitans* (Fig. 3). Seven of the F1 hybrids are males and only two are females, four males have *A. g. clamitans* female parental and *A. caraya* male parental and three males have the opposite parental combination. The two putative females descended of the former parental combination. Despite the small sample size, these data, together with the absence of mtDNA introgression but high Y-chromosome introgression, clearly suggested that female F1 hybrids are infertile or inviable while the two types of male hybrids are similarly viable and fertile (Fig. 6). These results suggested that male hybrids would likely

backcross with both purebred parental species. Actually, analyzing the distribution of the proportion for *A. g. clamitans*component in the non-F1 hybrids, four have values between 0.75 and 0.77, very similar to the 0.75 expect for F2 derived from backcrosses of F1 hybrids with pure *A. g. clamitans*, three have values between 0.83 and 0.89, similar to the 0.875 expected from F3 hybrids that are crosses between the F2 hybrids and pure *A. g. clamitans* (Fig. 3, Table S4).

Discussion

Here we show the utility of using the target capture UCE method on non-invasive samples. First, because it is an important genetic source for endangered species. Second, especially for herbivorous it is a good method to generate NGS data because it avoids contamination from other organisms on the diet (capturing only tetrapod's DNA). Third, because it allowsthe comparison for the same regions on the genome (i.e.: in different *Alouatta* species)..

The fecal samples studied here, although collected fresh, presented small quantity of endogenous DNA, as expected and observed inherbivorous animals (Perry *et al*. 2010).This suggested that the UCE target capture approach with probes designed to select ~three thousands ofendogenous loci (vertebrate) DNAmay be more appropriate for this kind of sample.

Here we confirm with thousands of genetic markers our previous genetic evidence of hybrids between these two species in this area (Mourthe *et al*., in prep.), also supporting previous observations of physically intermediate individuals and mixed bands (e.g. Bicca-Marques *et al*. 2008). We were able to show the occurrence of F1 hybrids in a narrow contact zones, but also found evidence of hybrid individuals that likely originated from serial backcrosses of hybrid males with purebred *A. g. clamitans* females. There is no evidence of any maternal (mtDNA) introgression, and only two female F1 hybrids, so female F1 hybridsseem inviable or infertile.

On the other hand, we found bidirectional introgression of the Y-chromosomes at least tens of kilometers away from both sides of the contact zone, on otherwise apparently purebred individuals (Fig. 3).In some cases, the hybrids can even live more than their parental species (Svedin et al. 2008; Grant and Grant 1996, 2010). Our results suggestthat male hybrids not only are fertile and may even have more reproductive success than purebred males (as seen in other species pair, Cortez-Ortiz *et al*. 2007).However, we still do not know about the difference of survival and lifespan between hybrids and parental species. This obscure our understanding of the importance of particularpre- and post-zygotic barriers, and thus, the true natureof hybrid disadvantage (Neubauer *et al.* 2014).

Interesting, previous molecular phylogenetic analyses showed that *A. caraya* and *A. g. clamitans* are not sister species and results based on mtDNA suggest they diverged approximately 5 million year ago (Cortés-Ortiz *et al.*2003), making this introgression at least unexpected. As suggested by previous morphological observations (reviewed in Cortez-Ortiz *et al*. 2015), and contrarily to the *A. pigra* x *A. palliata* hybrid zone, Haldane´s rule is not operating in A. *caraya* X *A. g. clamitans* system.

The Haldane's rule (Haldane 1922)considers that in a hybridization process the F1 offspring may have a fitness reduction of the heterogametic sex (males in mammals), which is an important mechanism of speciation in a variety of organisms (Coyne and Orr 2004). The introgression rates are substantial to maintain the hybrid zone and are determined by the ratio between the hybrid fitness and their parental species, combined with the frequency of admixture (Borges et al. 2005). To understand the fitness of the individuals involved in this system is required to know the number of viable offspring produced during a lifetime, which depends on annual survival probability (Neubauer et al 2014), and this data we still do not know.

Another important aspect that makes this hybridization a very special phenomenon, is the different number of sexual chromosomes between the species involved.*Alouatta* presents a

multiple sex chromosome system (the exception in Atelidae XX/XY), varying the number interspecific. *Alouatta guariba clamitans*presentsmultiple sexchromosome system $X_1X_1X_2X_2X_3X_3/X_1X_2X_3Y_1Y_2$and *A. caraya* presents $X_1X_1X_2X_2/X_1X_2Y_1Y_2$(Steinberg *et al.* 2014). The hypothesis suggested by Steinberg *et al.*(2014) for the evolution of this system consider that the *Alouatta* ancestor has a chromosomalsex determination XX/XY, before a biogeographic separation of Mesoamerican andSouth American groups.After this separation, both groups independentlydeveloped multiple sex chromosome systems. The sex chromosome system $X_1X_1X_2X_2/X_1X_2Y$ may have arisenseparetelyin the lineages of Meso and South American howlers by a Y-autosome translocation(Steinberg *et al.* 2014).

The multiple sex chromosome systems are an extremelyrare phenomenon in mammals (i.eg.: platypus and echidna - Bick and Jackson 1967, Renz *et al.* 2007) due to complication in meiosis. In primates, multiple sex chromosome systems are even more infrequent.Moreover, *Alouatta* would be the first case where an independent origin of multiple sexchromosome systems is described. More studies are necessary to understand how hybridization would be acting in this aspect.Our results suggest the present-day picture may represents the outcome of at least two or three different moments of contact between the two species. First, the *A. g. clamitans*Y chromosome introgression, in areas that are far away the contact zone, and in A. *caraya* individuals that present no trace of *A. g. clamitans* markers in the autosomal genome, is a signal of a hybridization at least several generations ago. In fact, as reviewed by Cortéz-Ortiz *et al*. (2015), in the Rio Grande do Sul State there are records of mixed groups since the beginning of the nineteenth century and reports of hybrids in museum specimens collected in the 1940s in the Parana State. On the other hand, in the contact area studied here, there are only F1 hybrids and a single F2 backcross, suggesting the contact here is very recent, at most two generations ago. Finally, the hybrids in the *A. g. clamitans* zone that seem at least F3 more hybrids may represent a hybridization that occurred at a time intermediate between the two described above.

The nearest *A. caraya* population recorded is at least 72 km away from this area (Silva and Codenotti 2007).

Introgression of genetic loci in areas away from the contact zone, as observed mainly for the presence of *A. caraya* Y-chromosomes in the *A. g. clamitans* zone and in otherwise purebred individuals, may be explained by phenomena such as some selective advantage on this chromosome or for the hybrid, or neutral demographic dynamics, or a combination of these. Similarly, the asymmetric nature of the hybridization on the autosomal genome, from *A. caraya* to *A. g. clamitans* (Fig. 3 and 4) could be explained by asymmetry in reproduction and viability and the above hypotheses. At the present, we could not test selective hypotheses but could speculate on possible demographic scenarios that would result in the observed picture. For example, in the latter case (the presence of *A. caraya* component in the *A. g. clamitans* zone), a simple scenario would be one in which *A. caraya* temporarily advanced into the present day exclusive *A. g. clamitans* zone, perhaps caused by a temporarily population reduction in *A. g. clamitans*, hybridized for very few generations, then retreated (given a population recovery on *A. g. clamitans*) to the previous distribution, leaving the (male) hybrids to cross for a few generations with mostly purebred *A. g. clamitans*. Although fast and temporarily shifts in distribution and changes population sizes may be natural phenomena, another explanation here may be the consequences of the yellow fever outbreaks in the region. Yellow fever outbreaks were periodic and seem to cause high rates of deaths in both species, and there is evidence that this have been occurring for at least several decades in the area (Almeida *et al*. 2012). The spread of the yellow fever is not homogeneous (Almeida *et al*. 2012) and some suggested that *A. g. clamitans* seems even more susceptible than *A. caraya* (J.C Bicca-Marques, pers. comm.), while the latter seems more plastic in habitat requirement (Holzmann *et al*. 2014). The above demographic scenario may have occurred in a previous yellow fever outbreak in the region. A similar, but older and in the other direction, scenario may explain the *A. g. clamitans* Y

chromosome introgressed in *A. caraya* zone, whether caused by yellow fever outbreaks or other natural causes.

Conclusions

To our knowledge, our study was the first to use the NGS approach to generate a large number of genetic markers to study genomic introgression in a contact zone using non-invasive samples, at least in Primates. The sequencing of Ultraconserved Elements in endogenous DNA from non-invasive samples is still challenging but we were able to produce very informative results even with a large number of missing data in several individuals. The Ultraconserved Elements has the advantage to be replicable, allowing comparison of the same regions on the genome with other hybrid zones of *Alouatta*in other settings (e.g., natural vs. anthropogenic, fragmented landscapes or continuous forest).

A more comprehensive understanding of genetic and phenotypic variation patterns in living hybrids populations as well as a detailed investigation of how long traits associated with hybridization may persist in the descendants are needed (Ackerman *et al*. 2010). Molecular analyses using large amount of independent loci are becoming even common nowadays and should be employed to confirm hybridization as well as to study more specific aspects of population structure in the hybrid zones. Our results show that using non-invasive sampling is a valid approach to study these phenomena. Genetic and behavioral studies in different primate's hybrids zones may offer knowledge about genetic composition, especially of individuals with reproductive success and could explain genetics effects, social dynamics and fitness of hybrids vs. pure individuals.

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Figures

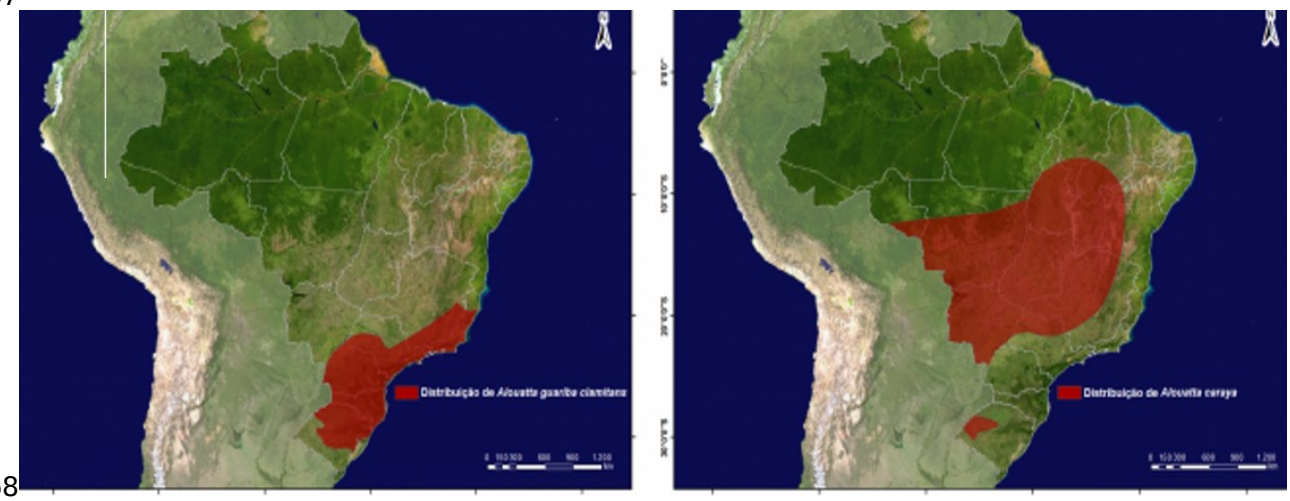


Fig. 1. Maps of distribution of *A. g. clamitans* (left) and *A. caraya*(right). Maps from Instituto Chico Mendes de Conservação da Biodiversidade (ICMBIO).

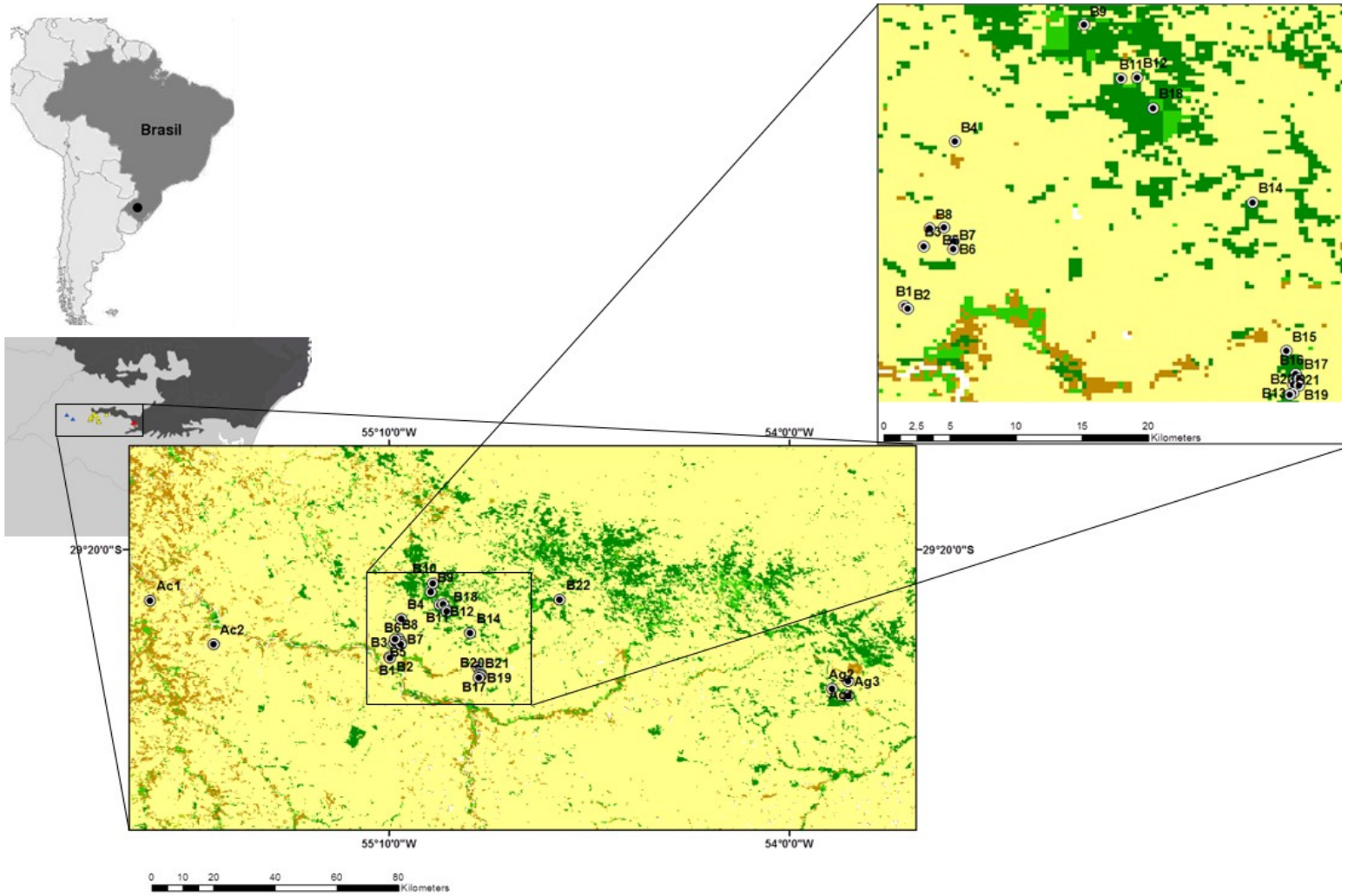


Fig 2: Geographic distribution of groups. Ac: *A. caraya* parental groups; Ag: *A. g. clamitans* parental groups; B: groups collected in the contact zone.

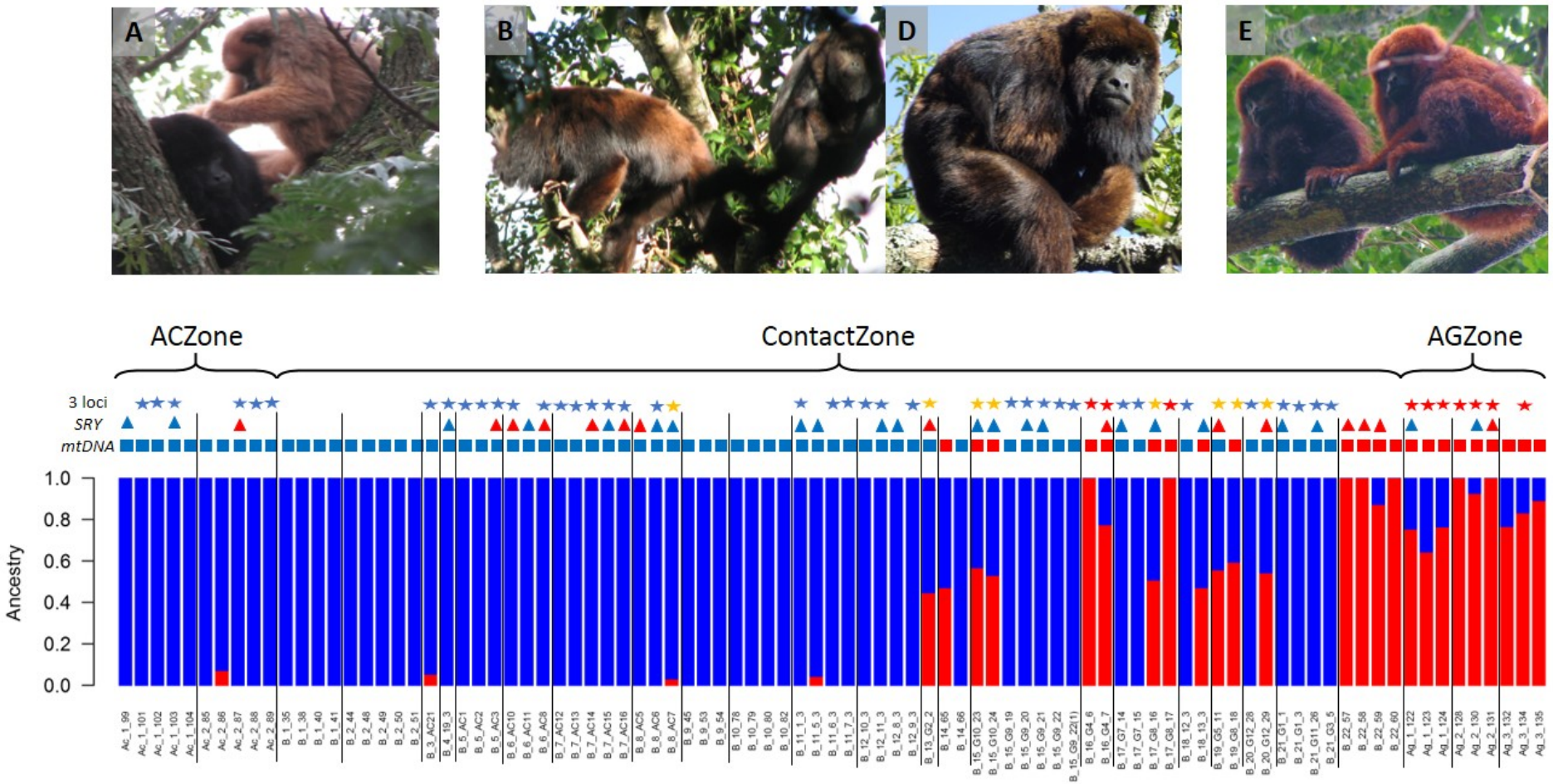


Fig. 3: ADMIXTURE proportion for K=2 for the filtered data set, clusters with prior population data not used. Populations are ordered by latitude. The squares indicate the mitochondrial (*mtDNA*) information for each sample and the triangles the fragment of the Y-chromosome (*SRY*) information (Blue: AC; Red: AG). The stars indicate the definition from the three microsatellite loci (Blue: *A. caraya*; Red: *A. g. clamitans*; Green: hybrid) analyzed by Mourthé *et al*. (in prep). (A) *A. caraya* pair – male: black; female: whitish to yellowish-buff (B) Hybrid individuals with mosaic pelage (Credits: Helissandra Prates and João Claudio Godoy) (C) *A. g. clamitans* pair – male:dark rufous to yellowish rufous dorsally female: dark brown or reddish brown hair.

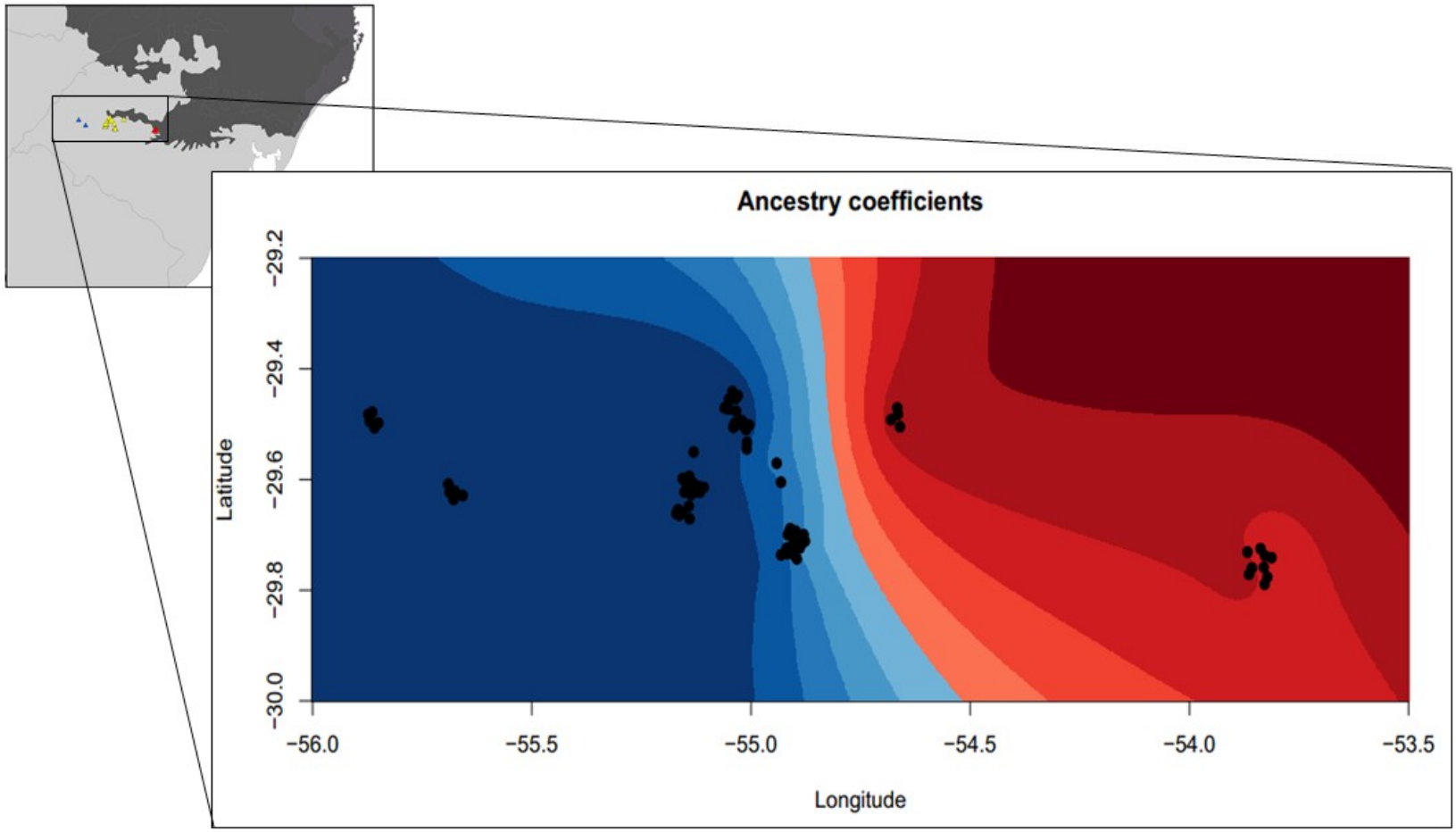


Fig. 4: Genetic structure of ACZone (blue scale), AGZone (red scale) and ContactZone (central colors)individuals as predicted by geographical covariates, results based on STRUCTURE (see Methods above).

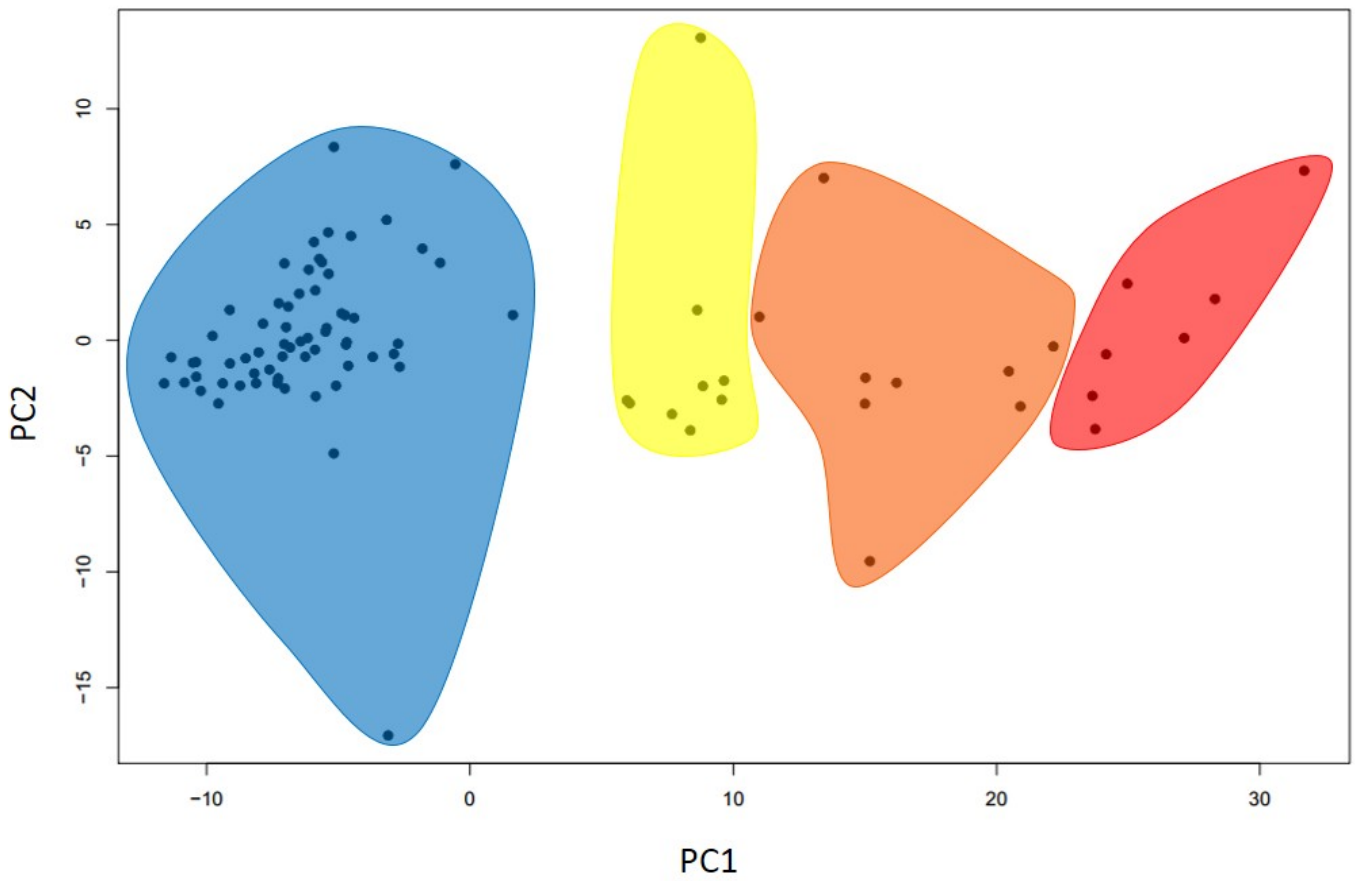


Fig. 5: Principal component analysis of individual *A. caraya*and *A. g. clamitans* genotypes. First and second axes representthe first two principal components. Pure AC individuals in blue cluster, pure AG individuals in red, F1 hybrids in yellow and AG admixture individuals in orange cluster.

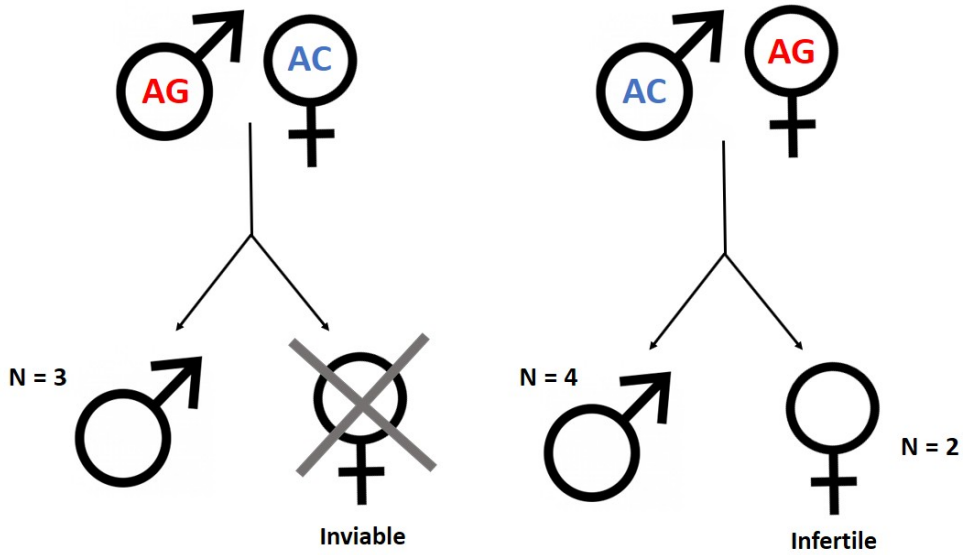


Fig 6: Possible outcomes of crosses between *A. caraya*(AC in blue) and *A. g. clamitans*(AG in red) males and females, according to thespecific identification of mitochondrial, Y-chromosome fragment and UCEs results, based on first generation. See text for explanation.

Tables

Table 1.Summary statistics for each zone collected including number of individuals sampled, raw of reads, average per individual, number of high quality reads (trimmed reads), number of reads with UCE loci mapped and percentage of reads used (with UCE loci mapped)

	NUMBER OF SAMPLES	TOTAL OF READS	AVERAGE PER INDIVIDUAL	NUMBER OF TRIMMED READS	AVERAGE PER INDIVIDUAL	READS WITH UCE LOCI MAPPED	AVERAGE PER INDIVIDUAL	% OVERALL OF RAW READS USED
AC ZONE	10	9579341	754838	8401655	726514	755450	23409	7.9
CONTACT ZONE	70	75097815	997619	72109864	919441	3711281	21763	4.9
AG ZONE	9	9614527	363628	6926785	362716	706307	4731	7.3

Supplementary Material

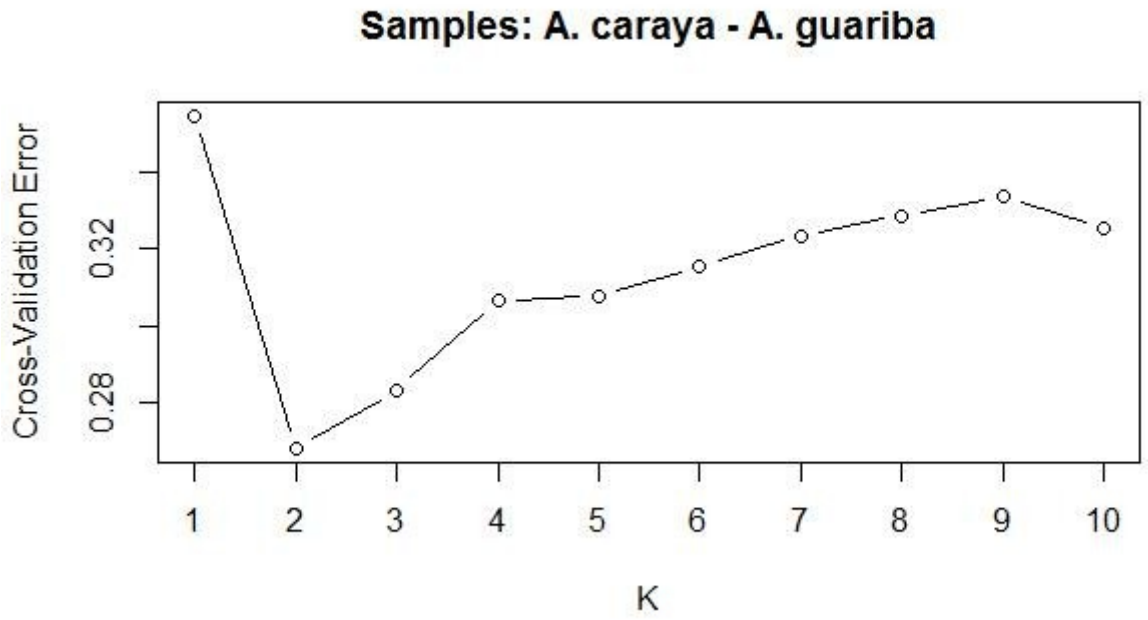


Fig. S1: Cross-validation plot for ADMIXUTRE from K=1 to K=10.

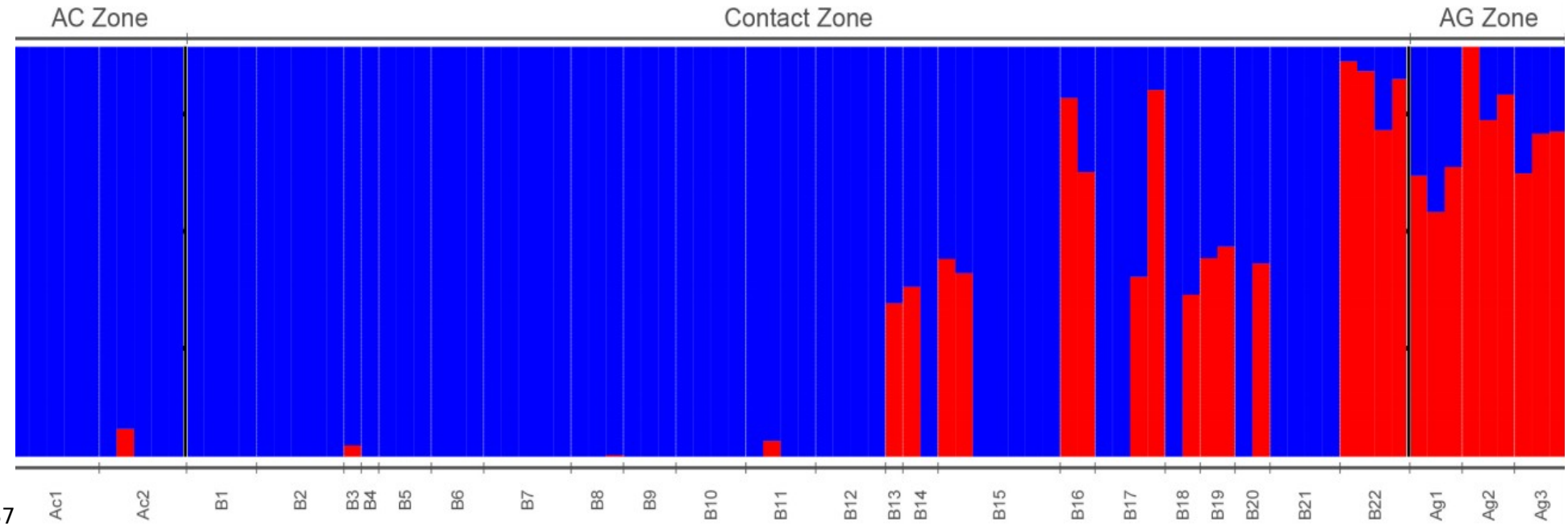


Fig. S2: Barplot of membership coefficient (qi) from STRUCTURE. K set for two populations. for the filtered data set. This barplot shows the groups composition in each sample zone. Populations are ordered by latitude; ACZone (groups collected as parental samples for *A. caraya*); ContactZone (groups collected within the contact zone); AGZone (groups collected as parental samples for *A. g. clamitans*).

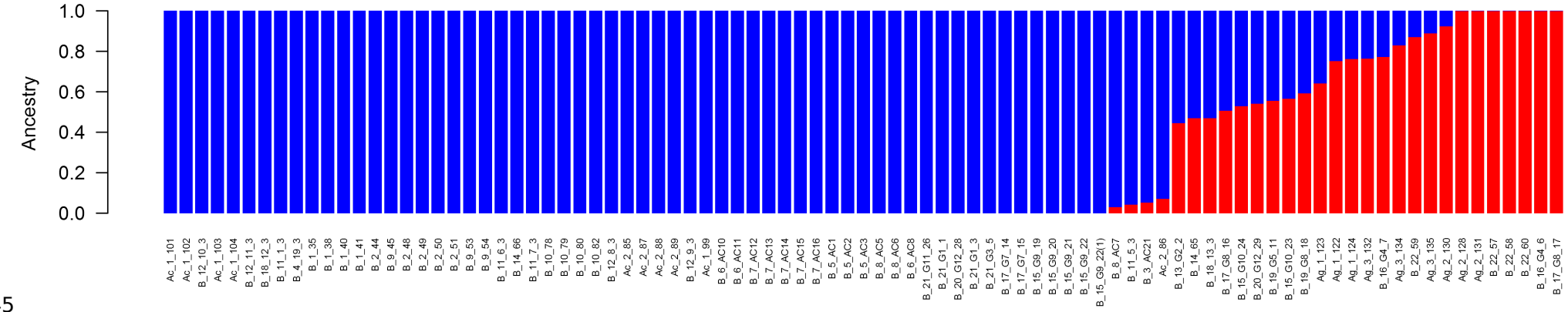
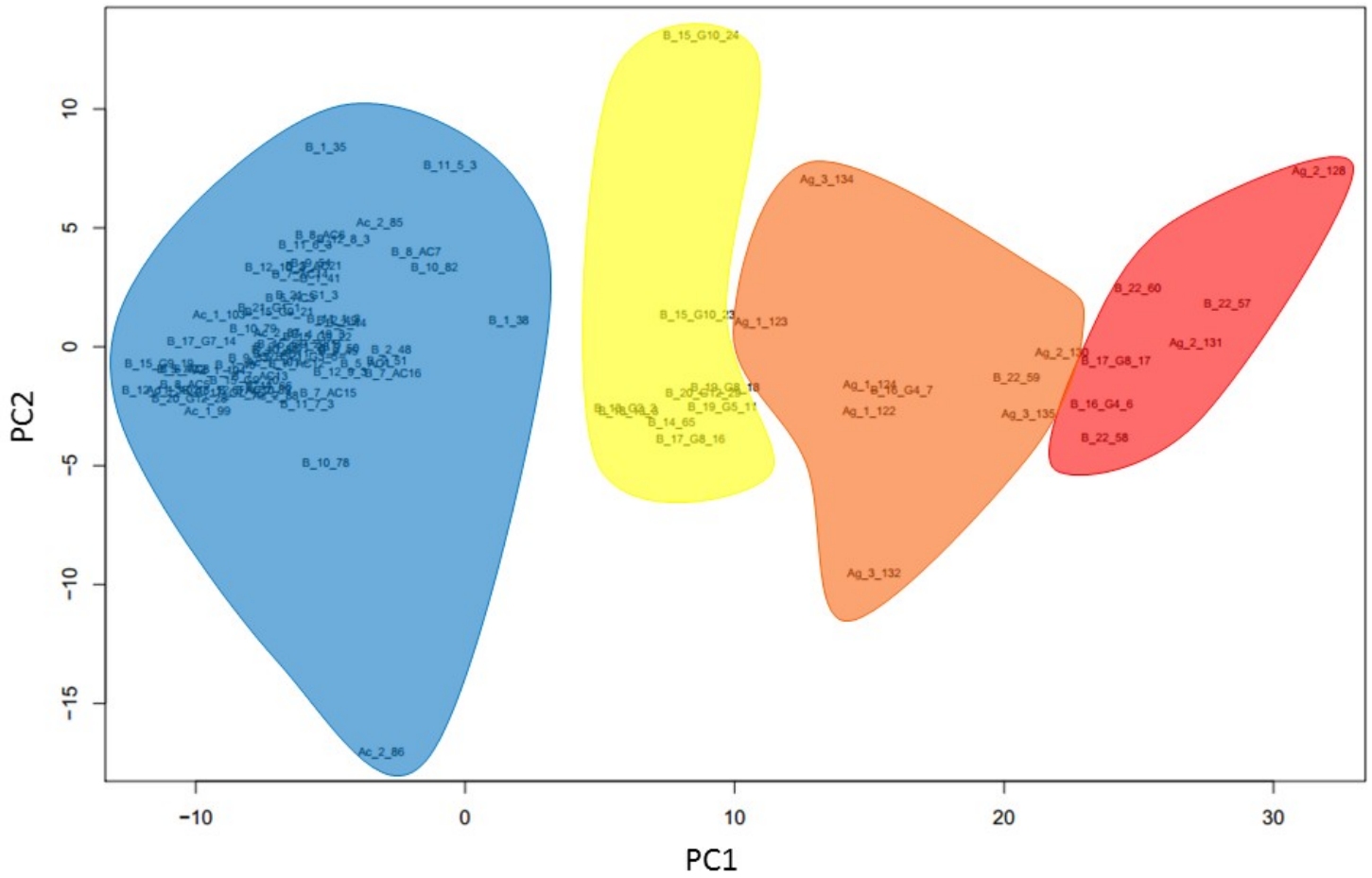


Fig. S3

: ADMIXTURE plor for all individuals, for the optimal number of genetic clusters (K=2), with ancestry proportion (Q) on the y-axis. The plot is for 3302 UCEs loci grouped by species. *A. caraya* clusters is in blue and *A. g. clamitans* is in red.


B_15_G10_24
B_1_35
B_11_5_3
Ac_2_85
B_8_AC7
B_10_82
B_1_38
B_2_48
B_7_AC16
B_7_AC15
Ac_1_99
B_10_78
Ac_2_86
Ag_3_134
Ag_1_123
Ag_1_124
Ag_1_122
Ag_3_135
Ag_3_132
B_22_59
B_16_G4_7
B_19_G5_11
B_14_65
B_17_G8_16
Ag_2_128
B_22_60
B_22_57
Ag_2_131
B_17_G8_17
B_16_G4_6
B_22_58
PC2
PC1
10
5
0
−5
−10
−15
−10
0
10
20
30

S4: Fig. 4: Principal component analysis of individual *A. caraya*and *A. g. clamitans* genotypes. First and second axes representthe first two principal components. Pure AC individuals in blue cluster, pure AG individuals in red, F1 hybrids in yellow and AG admixture individuals in orange cluster.

Table S1. Location of each fecal sample (UTM: Zone 21J: Ac1_99 - B22_60; Zone 22J: Ag1_122 - Ag3_135)

IDSample	Coord X	Coord Y	IDSample	Coord X	Coord Y
Ac1_99	610329	6736556	B11_7_3	692019.9	6734184.4
Ac1_101	610329	6736556	B12_10_3	693078.9	6734218.3
Ac1_102	610329	6736556	B12_11_3	693078.10	6734218.4
Ac1_103	610329	6736556	B12_8_3	693078.11	6734218.5
Ac1_104	610329	6736556	B12_9_3	693078.12	6734218.6
Ac2_85	628060	6722033	B13_G2_2	702838	6710703
Ac2_86	628060	6722033	B14_65	700479	6724585
Ac2_87	628060	6722033	B14_66	700479	6724585
Ac2_88	628060	6722033	B15_G10_23	702500	6713269
Ac2_89	628060	6722033	B15_G10_24	702500	6713269
B1_35	677535	6717068	B15_G9_19	702500	6713269
B1_38	677535	6717068	B15_G9_20	702500	6713269
B1_40	677535	6717068	B15_G9_21	702500	6713269
B1_41	677535	6717068	B15_G9_22	702500	6713269
B2_44	677758	6716835	B16_G4_6	703025	6711482
B2_48	677758	6716835	B16_G4_7	703025	6711482
B2_49	677758	6716835	B17_G7_14	703226	6711091
B2_50	677758	6716835	B17_G7_15	703226	6711091
B2_51	677758	6716835	B17_G8_16	703226	6711091
B3_AC21	678940.9	6721622.8	B17_G8_17	703226	6711091
B4_19_3	681103.4	6729605.8	B18_12_3	694068	6731950.1
B5_AC1	680239.6	6723077.1	B18_13_3	694069	6731950.2
B5_AC2	680239.7	6723077.2	B19_G5_11	703230	6710619
B5_AC3	680239.8	6723077.3	B19_G8_18	703230	6710619
B6_AC10	680845.3	6721921.8	B20_G12_28	702769	6710078
B6_AC11	680845.4	6721921.9	B20_G12_29	702769	6710078
B6_AC8	680845.5	6721921.10	B21_G1_1	702662	6709937
B7_AC12	680785.1	6721356.1	B21_G1_3	702662	6709937
B7_AC13	680785.2	6721356.2	B21_G11_26	702662	6709937
B7_AC14	680785.3	6721356.3	B21_G3_5	702662	6709937
B7_AC15	680785.4	6721356.4	B22_57	725991	6735157
B7_AC16	680785.5	6721356.5	B22_58	725991	6735157
B8_AC5	679292.2	6722991	B22_59	725991	6735157
B8_AC6	679292.3	6722992	B22_60	725991	6735157
B8_AC7	679292.4	6722993	Ag1_122	226432	6707191
B9_45	689614	6738374	Ag1_123	226432	6707191
B9_53	689614	6738374	Ag1_124	226432	6707191
B9_54	689614	6738374	Ag2_128	221800	6704654
B10_78	690124	6741150	Ag2_130	221800	6704654
B10_79	690124	6741150	Ag2_131	221800	6704654
B10_80	690124	6741150	Ag3_132	226402	6702393
B10_82	690124	6741150	Ag3_134	226402	6702393
B11_1_3	692019.6	6734184.1	Ag3_135	226402	6702393
B11_5_3	692019.7	6734184.2	Ag3_135	226402	6702393

B11_6_3	692019.8	6734184.3

Table S2.Summary of descriptive statistics for samples, Illumina sequencing and UCE loci.

IDSample	Total Reads	Trimmed Reads	UCEsMapped Reads	IDSample	Total Reads	Trimmed Reads	UCEsMapped Reads
Ac1_99	413.100	404566	69216	B11_7_3	926.240	923863	5069
Ac1_101	1.326.880	984895	23696	B12_10_3	828.166	825330	15757
Ac1_102	2.541.991	2021104	416982	B12_11_3	3.135.820	3128205	626125
Ac1_103	1.375.889	1169721	129591	B12_8_3	777.174	774435	26202
Ac1_104	428.859	425844	65730	B12_9_3	170.228	163811	15904
Ac2_85	232.241	227960	1865	B13_12_3	1.019.539	1016935	68974
Ac2_86	934.870	932664	1179	B13_13_3	231.353	230850	21520
Ac2_87	470.674	467196	13775	B14_65	1.122.592	940635	32285
Ac2_88	1.280.031	1247341	10295	B14_66	868.725	862311	15991
Ac2_89	574.806	520364	23121	B15_G10_23	536.269	533500	90282
B1_35	1.186.091	1158864	3463	B15_G10_24	1.425.443	1414313	3478
B1_38	1.191.891	390003	373	B15_G9_19	2.069.314	2060561	300126
B1_40	865.827	863855	90932	B15_G9_20	1.155.785	1152679	70702
B1_41	932.994	855345	11290	B15_G9_21	1.968.760	1956649	59860
B2_44	1.249.971	1248895	2797	B15_G9_22	523.688	516206	9489
B2_48	1.106.052	1103750	3871	B15_G9_22(1)	769.886	768631	24584
B2_49	173.597	161656	1517	B16_G4_6	1.651.834	1642767	48029
B2_50	1.370.626	1362459	11998	B16_G4_7	1.711.770	756877	12808
B2_51	466.679	466225	3578	B17_G7_14	836.925	836088	84182
B3_AC21	1.112.443	1073582	1492	B17_G7_15	717.832	699500	63759
B4_AC12	1.073.984	1069449	20343	B17_G8_16	1.761.371	1721240	80619
B4_AC13	653.323	646576	28376	B17_G8_17	703.549	624005	53880
B4_AC14	459.224	362729	15128	B17_G8_18	2.090.471	2086507	302540
B4_AC15	1.669.662	1658250	15503	B18_G2_2	264.489	262371	16108
B4_AC16	788.696	706318	4366	B19_G5_11	1.973.758	1952522	96551
B5_AC10	1.511.839	1506860	54505	B20_G12_28	2.256.484	2244012	90789
B5_AC11	1.106.606	1082543	23114	B20_G12_29	1.179.533	1171740	72769
B5_AC8	1.852.881	1849288	357506	B21_G1_1	691.562	687699	30650
B6_AC1	935.160	914631	3413	B21_G1_3	630.474	600583	14968
B6_AC2	774.645	756924	87165	B21_G11_26	1.216.918	1212586	22006
B6_AC3	886.998	884735	13929	B21_G3_5	1.422.492	1418358	18911
B7_AC5	1.592.895	1579712	144159	B22_57	814.519	813540	127611
B7_AC6	991.316	982744	3061	B22_58	558.724	557709	45300
B7_AC7	1.361.54	1344473	1691	B22_59	740.804	739375	23637

B8_19_3	1.003.920	1002387	2666	B22_60	345.331	344788	32009
B9_45	1.300.651	1298097	11567	Ag1_122	363.628	362716	4731
B9_53	1.110.766	1107768	47711	Ag1_123	3.649.042	1040438	794
B9_54	1.450.946	1241617	7235	Ag1_124	838.639	837822	3886
B10_78	1.052.164	1041737	12411	Ag2_128	2.442.948	2383356	497042
B10_79	759.108	758141	34835	Ag2_130	307.660	306786	34552
B10_80	920.713	915018	27829	Ag2_131	1.444.337	1438615	133976
B10_82	575.716	573989	1862	Ag3_132	172.529	171729	2310
B11_1_3	1.468.860	1466994	11926	Ag3_134	112.456	103133	1006
B11_5_3	436.551	435552	885	Ag3_135	283.288	282190	28010
B11_6_3	605.651	597517	25310				

Table S3: Missing data per individual

IDSample	Missing (%)	IDSample	Missing (%)
Ac_1_99	17	**B_11_6_3**	64
Ac_1_101	35	**B_11_7_3**	80
Ac_1_102	9	**B_12_10_3**	56
Ac_1_103	26	**B_12_11_3**	3
Ac_1_104	19	**B_12_8_3**	69
Ac_2_85	85	**B_12_9_3**	74
Ac_2_86	93	**B_13_G2_2**	47
Ac_2_87	55	**B_14_65**	33
Ac_2_88	66	**B_14_66**	50
Ac_2_89	43	**B_15_G10_23**	38
B_1_35	88	**B_15_G10_24**	88
B_1_38	96	**B_15_G9_19**	7
B_1_40	28	**B_15_G9_20**	26
B_1_41	73	**B_15_G9_21**	45
B_2_44	83	**B_15_G9_22**	69
B_2_48	82	**B_15_G9_22(2)**	48
B_2_49	89	**B_16_G4_6**	26
B_2_50	66	**B_16_G4_7**	54
B_2_51	77	**B_17_G7_14**	17
B_3_AC21	88	**B_17_G7_15**	24
B_4_AC12	51	**B_17_G8_16**	19
B_4_AC13	40	**B_17_G8_18**	5
B_4_AC14	56	**B_17_G8_17**	28
B_4_AC15	57	**B_18_12_3**	21
B_4_AC16	86	**B_18_13_3**	40
B_5_AC10	25	**B_19_G5_11**	19
B_5_AC11	40	**B_20_G12_28**	21
B_5_AC8	12	**B_20_G12_29**	19
B_6_AC1	78	**B_21_G1_1**	36
B_6_AC2	15	**B_21_G1_3**	57
B_6_AC3	56	**B_21_G11_26**	50
B_7_AC5	15	**B_21_G3_5**	49
B_7_AC6	79	**B_22_57**	14
B_7_AC7	87	**B_22_58**	43
B_8_19_3	85	**B_22_59**	43
B_9_45	73	**B_22_60**	50
B_9_53	33	**Ag_1_122**	74
B_9_54	66	**Ag_1_123**	92
B_10_78	64	**Ag_1_124**	78
B_10_79	38	**Ag_2_128**	2
B_10_80	38	**Ag_2_130**	29
B_10_82	88	**Ag_2_131**	10
B_11_1_3	64	**Ag_3_132**	84
B_11_5_3	94	**Ag_3_134**	94
		Ag_3_135	32

Table S4: Distribution of proportion of each ancestral component (AG and AC).

IDSample	AG ancestry	AC ancestry	Definition
B_11_5_3	0.04	0.96	
B_3_AC21	0.05	0.95	AC
Ac_2_86	0.07	0.93	
B_13_G2_2	0.44	0.56	
B_14_65	0.47	0.53	
B_18_13_3	0.47	0.53	
B_17_G8_16	0.51	0.49	F1
B_15_G10_24	0.53	0.47	
B_20_G12_29	0.54	0.46	
B_19_G5_11	0.56	0.44	
B_15_G10_23	0.57	0.43	
B_19_G8_18	0.59	0.41	
Ag_1_123	0.64	0.36	?
A_g_1_122	0.75	0.25	
A_g_1_124	0.76	0.24	F2
A_g_3_132	0.76	0.24	
B_16_G4_7	0.77	0.23	
A_g_3_134	0.83	0.17	
B_22_59	0.87	0.13	F3
A_g_3_135	0.89	0.11	
A_g_2_130	0.92	0.08	F4
Ag_2_128	1.00	0.00	
A_g_2_131	1.00	0.00	
B_22_57	1.00	0.00	
B_22_58	1.00	0.00	AG
B_22_60	1.00	0.00	
B_16_G4_6	1.00	0.00	
B_17_G8_17	1.00	0.00	

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