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CELLULAR AND MOLECULAR BIOLOGY

Microsatellite Markers for Bearded Capuchins (*Sapajus libidinosus*): Transferability and Characterization

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Abstract: Natural *Sapajus libidinosus* populations are in continuous decline due to fragmentation, habitat loss, and the illegal pet trade. They live in Caatinga scrub forests, which already lost over 50% of their original cover. The lack of studies on *S. libidinosus* population genetics means that we do not know how they are being affected by this striking habitat loss and other anthropogenic disturbances. Polymorphic markers are not available for the study of *S. libidinosus* diversity and population genetics. Thus, here we aimed to test the transferability of 14 microsatellite markers to *S. libidinosus*. These microsatellites were previously isolated from *Cebus capucinus* (white-faced capuchin), species belonging to the same subfamily (Cebinae) as the study species. We found that six of the tested microsatellite markers (tetra-nucleotide) were cross-amplified in our target species. All loci were polymorphic. The number of alleles varied from 4 to 7, and the expected heterozygosity ranged from 0.588 to 0.869. The microsatellite markers transferred to *S. libidinosus* and characterised in our study will be valuable tools to evaluate the genetic variability of both wild and captive populations. They will considerably reduce the costs of microsatellite isolations, helping to prioritise currently limited research and conservation budgets in Brazil.

Key words: Cross-amplification, DNA extraction, faecal samples, molecular markers, primates.

INTRODUCTION

Capuchin monkeys are classified into two distinct clades: tufted or robust capuchins (*Sapajus*) and the non-tufted or gracile capuchins (*Cebus*) (Lynch-Alfaro et al. 2012, Lima et al. 2018). The clades are differentiated by morphological, ecological and molecular characteristics (Lynch-Alfaro et al. 2012). The tufted form has specialized cranial and dental structures for the exploitation of hard foods items, and possess forelegs, hands and feet that are shorter than those of the non-tufted clade (Silva 2001, Lynch-Alfaro et al. 2012). Additionally, the tufted capuchins make use of tools to obtain hard-to-access food items such as underground (e.g. roots) or encapsulated resources (e.g. palm nuts, cashew nuts) (Moraes et al. 2014). At present, eight species of tufted capuchin monkeys are recognized: *Sapajus apella*, *Sapajus cay*, *Sapajus flavius*, *Sapajus libidinosus*, *Sapajus macrocephalus*, *Sapajus nigritus*, *Sapajus robustus*, and *Sapajus xanthosternos* (IUCN 2019). *Sapajus libidinosus* are Neotropical primates, distributed in Brazil throughout Cerrado areas in the central, north and south-eastern regions, and in Caatinga areas in the north-eastern region (Rylands & Kierulff 2015). Although the *S. libidinosus* is not classified as a Threatened species (Rylands &

Kierulff 2015), it has been reclassified as "Near Threatened" by the Brazilian Red Book of Endangered species, owing to anthropogenic effects such as habitat loss and fragmentation (Fialho et al. 2015, Rylands & Kierulff 2015). Such impacts directly influence the viability of primate populations that rely on forest habitats to survive (Benchimol & Peres 2013, Liu et al. 2017). Nevertheless, the lack of studies on population genetics in *S. libidinosus* limits our knowledge of how habitat loss is genetically affecting the species. Polymorphic markers are not yet available to study their diversity and population structure.

Microsatellite markers have been developed and tested on other capuchin monkeys such as *Sapajus apella* (Escobar-Páramo 2000), *Cebus capucinus* (Muniz & Vigilant 2008) and *S. nigritus* (Tokuda et al. 2014). The characterization of genetic markers is a matter of great importance for population genetics studies, which are urgently required to assess gene flow and long-term viability of populations (Liu et al. 2017, León-Ortega & Gonzalez-Wangüemert 2015, Tian et al. 2017, Srbek-Araujo et al. 2018). Microsatellite markers may also be useful in behavioural studies (Tokuda et al. 2018, Yewers et al. 2019, Hoogland et al. 2019). Such studies contribute to effective management and conservation strategies (Ruiz Lopez et al. 2016, Storfer et al. 2018).

This study will provide molecular markers that can be used in future genetic studies on *Sapajus libidinosus* which need an urgent consideration in research and conservation agendas for the species and its habitat (e.g. Lynch-Alfaro et al. 2014). Using primers transferability has become a more economical and efficient way of obtaining genetic information from species when compared to developing new primers (Oliveira et al. 2006, Buzatti et al. 2016). Thus, here we aimed to test for amplification and characterize 14 microsatellite loci in *S. libidinosus*. These microsatellites were previously isolated from *Cebus capucinus* (Muniz & Vigilant 2008), which belongs to the same subfamily (i.e. Cebinae) of the *S. libidinosus* (Groves 2016). The molecular markers tested here will help to advance conservation actions and genetic management of *S. libidinosus* populations.

MATERIAL AND METHODS

Sampling

We sampled individuals of *Sapajus libidinosus* from two localities in Sertânia, Pernambuco state, north-eastern Brazil: 1) Serra do Pinheiro: -37.20 w, -8.38 s; 2) and in Serra do Estrago: -37.40 w, -7.96 s (Figure 1). We collected 22 faecal samples in September of 2016 in the Serra do Estrago and 27 faecal samples in December 2016 in Serra dos Pinheiros. In each study area, we established food provision stations in order to attract the animals and consequently facilitate the collection of faeces. We monitored the provision stations using camera traps (model Bushnell 8MP) to confirm species identity as we did not habituate the animals (Figure 2). We also performed active searches in both areas to find the animals when they did not access the provision stations. We stored the faeces in Falcon tubes containing 50 ml of absolute alcohol in the field and then we froze them (-18° C) in the laboratory for up to 12 months prior to DNA extraction (Serra do Pinheiro samples were frozen for 12 months and Serra do Estrago samples for 9 months). Three blood samples from *S. libidinosus* individuals were used to ensure the right allele size in genotyping. We obtained two of those samples from free-living individuals from the municipality of Serra Talhada, Pernambuco, Brazil with the help of the National Center for Research and Conservation

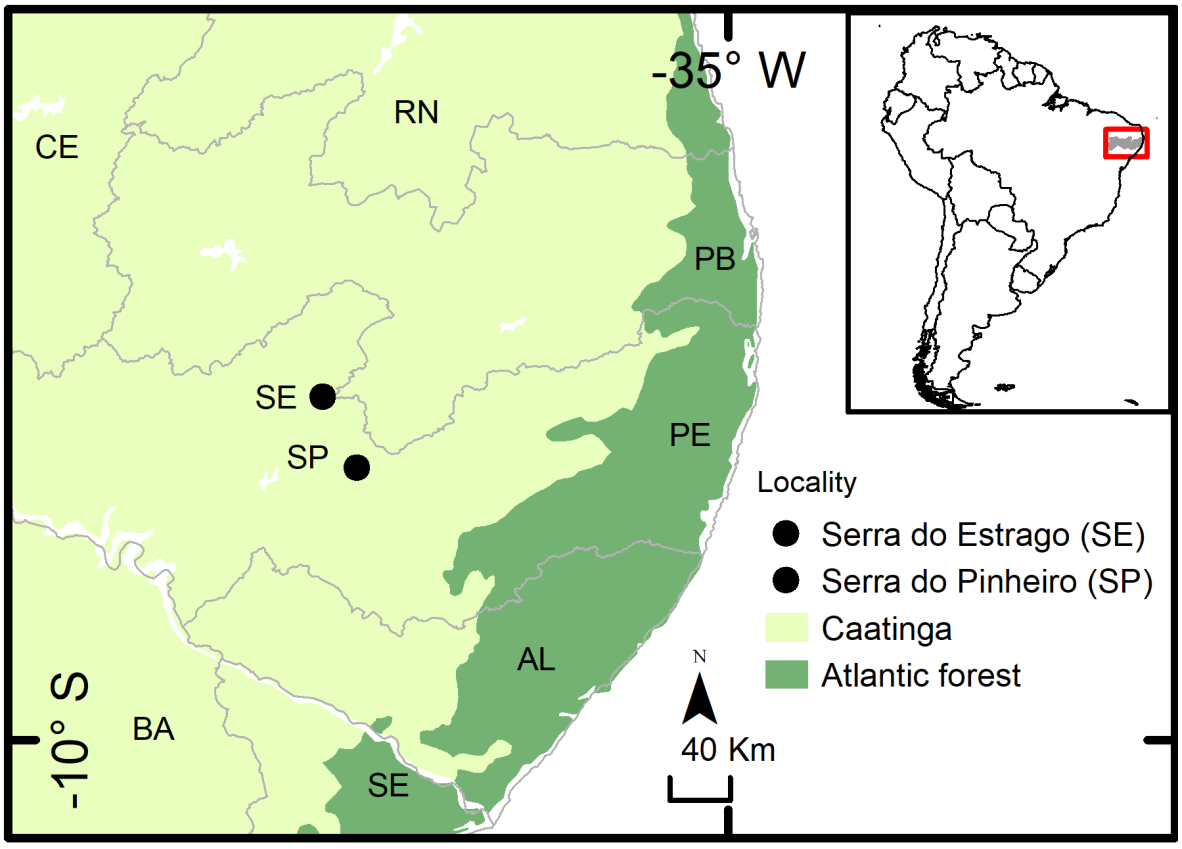

Figure 1. Location of the *S. libidinosus* populations sampled in the present study. The populations live in fragments of Caatinga Biome, in the state of Pernambuco, North-eastern Brazil. Serra do Pinheiro (SP): -37.20 w, -8.38 s, Serra do Estrago (SE): -37.40 w, -7.96 s. (States: CE-Ceará; RN- Rios Grande do Norte; PB- Paraíba; PE- Pernambuco; AL - Alagoas; SE- Sérgio; BA- Bahia).

of Brazilian Primates (CPB). We obtained the third sample from a captive individual located at the Dois Irmãos State Park Zoo in Recife, Pernambuco, during the routine health check-up of the individual. We kept the blood samples frozen before analysis.

DNA extractions

We extracted the DNA from blood samples using a QIAamp DNA Mini Kit (Qiagen), following the QIAamp® DNA Mini and Blood Mini Handbook protocol for DNA Purification from Blood or Body Fluids (Spin Protocol).

DNA from faecal samples was extracted using a QIAamp DNA stool kit (Qiagen), specifically developed for faecal DNA purification (Di Fiore 2003, Bradley et al. 2007). We used extraction protocols provided by Qiagen kits along with the adjusted protocols proposed by Di Fiore et al. (2009) and Tokuda (2012). Briefly, the changes we made in the protocols were: 1) the DNA was extracted from approximately 100mg of faeces; 2) faecal samples were incubated at room temperature in ASL buffer for 30 to 60 minutes, instead of 10 min; 3) the samples were incubated with proteinase K at 70 °C for 30 minutes, instead of 10 minutes; and 4) we added 100 µl of buffer AE and samples were incubated at room temperature for 20 to 30 minutes, instead of 200 µl for 1 minute. The modified protocol was used for the samples with a low concentration of *Sapajus libidinosus* DNA.

Figure 2. Camera trap image confirming the species identity. The image shows a group of *S. libidinosus* at Serra do Estrago, Pernambuco, Northeast Brazil.

Microsatellite amplification and analysis

We tested the transferability to *Sapajus libidinosus* of 14 microsatellites (Ceb01, Ceb02, Ceb04, Ceb07, Ceb08, Ceb09, Ceb10, Ceb105, Ceb115, Ceb119, Ceb120, Ceb121, Ceb127, Ceb130: Muniz & Vigilant (2008) developed for *Sapajus capucinus* and already tested for transferability on other Neotropical primate species (see Muniz & Vigilant 2008, Tokuda et al. 2014). The markers were tested in two steps. In the first, we used DNA extracts from the blood samples to test the amplification temperature gradient of each marker. The temperature gradient tested was between 54°C and 64°C. In each set of samples, we used negative controls to make sure the material was not contaminated. After identifying which markers amplified the blood DNA extracts, their annealing temperature and allele size, we tested these markers on stool DNA samples from *S. libidinosus* individuals from two localities. The Polymerase Chain Reactions (PCRs) mix contained 13 µL reaction volume and consisted for 2 µL DNA (5-63 ng/µL), 7.5 µL (2x) MyTaqMix (Bioline), 1.2 µL (6mg) bovine serum albumin, 0.3 µL (10µ/m) forward primer, 0.3 µL (10µ/m) reverse primer and 1.7 µL H_2O. We labelled each forward primer with the fluorochromes 6-FAM or HEX in the 5' end. Amplifications were carried out under the following conditions: an initial denaturation step at 95°C for 1 min; 35 cycles at 95°C for 1min, annealing at 56-64°C for 45 s, and extension at 72°C for 1 min; and a final extension for 10 min at 72°C. The difference between the PCR protocols of the blood and stool samples is that we used 1 µL of blood DNA extract and 2 µL for stool, adjusting only with the amount of water 2.7 µL and 1.7 µL, respectively. For both the blood and faeces samples, we used negative controls to make sure the material was not contaminated. For stool samples, we also used a positive control (i.e. blood DNA extract) to confirm the amplification of the marker. We

visualised amplified fragments on 2% agarose gels. We genotyped PCR products through Source BionScience (ABI Prism 3730), and allele sizes were scored using the program Geneious version 11.1.4 (Kearse et al. 2012).

We analysed the number of alleles per locus (N_A), observed heterozygosity (*H*o), expected heterozygosity (*H*e), probability of paternity exclusion (Q), identity index (*I*), polymorphic information content (PIC) and identity analysis, using CERVUS 3.0.7 software package (Kalinowski et al. 2007). We calculated the exact test of Hardy-Weinberg equilibrium using Arlequin v. 3.5.2.2 (Excoffier & Lischer 2010), and the fixation index (Fis) for each locus using the Fstat v. 2.9.3.8 (Goudet 2002), both analyses considering the Bonferroni correction of the confidence intervals (Rice 1989). To test for evidence of null alleles, we used the program MICRO-CHECKER (Van Oosterhout et al. 2004). We performed these analyses in two ways: the first, considering samples from each locality separately and the second, considering samples of both localities together.

RESULTS

We managed to amplify six of the 14 microsatellite markers used (Ceb 130, Ceb 120, Ceb 105, Ceb 10, Ceb 4, Ceb 7) using the blood samples (Table I). Subsequently, we tested the selected primers on the DNA extracted from the 49 faecal samples collected. We treated 11 faecal samples using the modified protocol for DNA extraction. Nine of those samples resulted in increased DNA concentration, demonstrating the effectiveness of the protocol. The remaining 38 samples were treated with the standard Qiagen protocol. Overall, we amplified the markers in 30 of the 49 samples collected, 15 in each locality. The mean proportion of amplified loci was 0.989 in Serra do Pinheiro, 0.956 in Serra do Estrago samples and 0.9722 considering the samples of both localities together. Ceb01, Ceb02, Ceb08, Ceb09, Ceb115, Ceb119, Ceb121, Ceb127 did not amplify in any sample. The number of alleles, the observed and the expected heterozygosities, the probability of paternity exclusion, the identity indexes and the polymorphic information contents are presented in Table I. No loci showed evidence of null alleles and the identity test showed that there are no repeated individuals in the sample. No locus showed a significant deviation from the Hardy-Weinberg equilibrium in Serra do Pinheiro, but in the Serra do Estrago, three loci presented a significant result Ceb130, Ceb120 and Ceb7 (Table I). When we analysed the samples of both localities together, the HWE was also significant for the Ceb130, Ceb120 and Ceb7 loci. The Fis values showed negative and significant values (*p* smaller) for some loci, indicating an excess of heterozygosity (Serra do Pinheiro: Ceb7; Serra do Estrago: Ceb120 and Ceb7; samples from both localities together: Ceb130, Ceb10 and Ceb4) (Table I).

DISCUSSION

We found that six of the 14 tested markers successfully amplified in the *Sapajus libidinosus* samples and thus, we can effectively use them when designing future population genetic studies for the species, considerably reducing the costs of microsatellite marker isolation. The absence of null alleles demonstrates that the genotyping performed in this study does not present errors such as non-amplified alleles, stutter peaks or short allele dominance (large allele dropout) (Van Oosterhout et al. 2004). All markers tested were tetranucleotides and markers of this type are considered more consistent for allele identification through automated techniques (Di

Table I. Genetic characterization, of the six microsatellite loci transferred to *Sapajus libidinosus* living in Serra do Pinheiro and Serra do Estrago in Sertânia municipality, Pernambuco State, north-eastern, Brazil. *N- number of individuals tested, Ta – annealing temperature, (NA)* Number of alleles, (*Ho*) observed heterozygosity, (*He*) expected heterozygosity, *(PIC)* polymorphic information content, (*Q*) probability of paternity exclusion, (*I*) identity index, (HWE) Hardy–Weinberg equilibrium (*p* = 0.0083 after Bonferroni correction) and (Fis) fixation index (p = 0.00341 for localities separately, and p= 0.0083 for single population after Bonferroni correction); [c] combined probability, *significant; ***p* smaller significant, indicating excess heterozygosity)

Serra do Pinheiro forest fragment												
Locus	**GenBank ID**	**Ta (°C)**	***N***	**Allele size range (pb)**	N_A	***Ho***	***He***	***PIC***	***Q***	***I***	**HWE**	**Fis**
Ceb130	EU019215	64	15	210 - 282	7	0.933	0.869	0.820	0.679	0.046	0.23354	-0.077
Ceb120	EU019211	64	14	248 – 278	7	0.643	0.815	0.759	0.596	0.073	0.35481	0.217
Ceb105	EU019208	64	15	228 - 240	4	0.733	0.697	0.620	0.419	0.160	0.62538	-0.055
Ceb10	EU019203	59	15	238 - 250	4	0.800	0.605	0.543	0.355	0.215	0.47371	-0.339
Ceb7	EU019200	64	15	123 - 175	7	1.000	0.768	0.713	0.541	0.096	0.10861	-0.317-
Ceb4	EU019199	54	15	166 - 214	5	0.867	0.602	0.519	0.327	0.238	0.14529	0.462
All loci	-	-	-	-	5.667	0.829	0.726	0.6621	0.9849[c]	0.000002[c]	-	-0.148**
Serra do Estrago forest fragment												
Locus	**GenBank ID**	**Ta (C)**	**N**	**Allele size range (pb)**	N_A	***Ho***	***He***	***PIC***	***Q***	***I***	**HWE**	**Fis**
Ceb130	EU019215	64	15	262 – 278	4	1.000	0.724	0.645	0.442	0.145	0.00009*	-0.400
Ceb120	EU019211	64	15	250 – 274	4	1.000	0.701	0.619	0.413	0.163	0.00165*	-0.448**
Ceb105	EU019208	64	15	224 – 272	5	0.667	0.653	0.580	0.384	0.188	0.23531	-0.022
Ceb10	EU019203	59	15	238 – 258	5	0.933	0.717	0.650	0.459	0.137	0.68687	-0.315
Ceb7	EU019200	64	13	127 – 167	4	1.000	0.618	0.515	0.315	0.244	0.00310*	-0.326**
Ceb4	EU019199	54	13	166 – 178	4	0.769	0.588	0.521	0.334	0.233	0.61242	-0.660
All loci	-	-	-	-	4.333	0.894	0.791	0.5883	0.9501[c]	0.000034[c]	-	-0.360**
Serra do Pinheiro + Serra do Estrago												
Locus	**GenBank ID**	**Ta (°C)**	**N**	**Allele size range (pb)**	N_A	***Ho***	***He***	***PIC***	***Q***	***I***	**HWE**	**Fis**
Ceb130	EU019215	64	30	262 – 278	7	0.967	0.810	0.768	0.604	0.070	0.00163*	-0.197**
Ceb120	EU019211	64	29	250 – 274	9	0.828	0.775	0.728	0.557	0.090	0.00708*	-0.069
Ceb105	EU019208	64	30	224 – 272	6	0.700	0.668	0.606	0.41	0.169	0.43228	-0.049
Ceb10	EU019203	59	30	238 – 258	5	0.867	0.666	0.618	0.433	0.156	0.19439	-0.308**
Ceb7	EU019200	64	28	127 – 167	8	1.000	0.819	0.777	0.618	0.065	0.00000*	-0.371
Ceb4	EU019199	54	28	166 – 178	6	0.821	0.603	0.549	0.364¶	0.209	0.25641	-0.226**
All loci	-	-	-	-	6.667	0.8638	0.7235	0.6744	0.9857*¶	0.000002*	-	-0.198**

Fiore 2003, Liu et al. 2008). Tokuda et al. (2014) tested eight (i.e. Ceb3, Ceb8, Ceb9, Ceb11, Ceb119, Ceb120, Ceb121, and Ceb130) of the primers developed for *Cebus capucinus* in 21 *S. nigritus* and only Ceb3, Ceb11 and Ceb130 amplified successfully. Despite amplifying successfully in *S. nigritus*, Ceb3 and Ceb11 loci showed only two alleles (Tokuda et al. 2014). On the other hand, Ceb130 showed a greater polymorphism with eight alleles for *S. nigritus* (Tokuda et al. 2014). Comparing the parameters of the Ceb130 locus, common to the three species tested, *C. capucinus* (Muniz & Vigilant 2008), *S. nigritus* (Tokuda et al. 2014) and *S. libidinosus* (present study), we found differences in annealing temperature and size of the alleles. The annealing temperature ranged from 59°C in *C. capucinus* (Muniz & Vigilant 2008), to 60°C in *S. nigritus* (Tokuda et al. 2014) and 64°C in *S. libidinosus*. The size of alleles ranged from 182-218 in *C. capucinus* (Muniz & Vigilant 2008) and 210-282 in *S. libidinosus*, and this information was not available for *S. nigritus*. Muniz & Vigilant (2008) developed primers for *C. capucinus* and tested the transferability of these primers to 23 Neotropical primate species. However, the number of individuals tested was very low in each species (only three species were tested using 10 individuals). Despite the low number of individuals, the species tested were polymorphic in at least two loci and species from the same family of *S. libidinosus* (i.e. *S. apella* n = 10 individuals, *C. olivaceus* n = 3 and *S. xanthosternos* n = 3) amplified the largest number of loci (Muniz and Vigilant, 2008), probably due to their phylogenetic proximity.

The obtained heterozygosity values indicate that the study localities of *Sapajus libidinosus* have a high genetic variation. The values of the paternity exclusion index (Q) in our study show the reliability of the loci for paternity test use (Wang et al. 2015). Studies previously conducted for other primates consider indexes around 0.9660 and 0.9999 as being powerful for paternity testing (Chambers et al. 2004, Stevanovic et al. 2010, Wang et al. 2015). In our study, these values varied among localities and showed good power of exclusion. The identity index indicates that the probability of identifying two different individuals as being of the same genotype is low, demonstrating the reliability of the studied sites for this type of estimation. Values between 0.01-0.0001 are sufficiently low and indicate that the chosen loci are effective to distinguish between individuals (Waits et al. 2001). One can also access genetic variability among genotypes by calculating PIC values for each of the six loci. The PIC value can range from 0 - 1.0, the closer to one, the greater the allele variability in a tested locality (Guo & Elston 1999). This value is calculated by considering the number of known alleles and their frequency of distribution (Botstein et al. 1980, Guo & Elston 1999). The PIC values indicated a high variability for the markers analysed here, in both our study sites. We found that the Ceb130 marker would be the most polymorphic marker followed by Ceb120, Ceb10 and Ceb4. Polymorphic markers are more useful for distinguishing individuals and understanding the relationships between them (Guo & Elston 1999, Li et al. 2014). In the Serra do Pinheiro, there was no deviation from the HWE. However, three loci deviated from the HWE in the Serra do Estrago (Ceb130, Ceb120 and Ceb7) and also when analysing the samples from both localities together. Deviation from HWE can be an outcome from heterozygosity excesses (Cornuet & Luikartt 1996), which was the case in our study. The species social system may have contributed to this result. The mating system of the study species is considered to be polygamous (Fialho et al. 2015), increasing the possibility of mating between the individuals of the group. They present a hierarchy of dominance maintained by males, but males disperse while females remain in the group (Verderane 2010).

The study locations presented approximately 25 individuals, a common size for groups of capuchin monkeys. The standard size of *S. libidinosus* groups is 6 to 20 individuals (Rylands & Kierulff 2015), although groups of 40 to 53 individuals have previously been recorded (Ferreira et al. 2009, Moraes et al. 2014).

It is worth pointing out that the DNA extracted from the faecal samples was "impure" primate DNA and despite the low concentrations (i.e. between 5 and 63 ng/µl), it was still possible to evaluate the efficiency of the primers tested. Some researchers recommend the multiple-tube approach in order to minimise the allelic dropout errors that may occur in low-concentration DNA from non-invasive samples (Gerloff et al. 1999, Kohn et al. 1999). However, due to few amounts of biological material and DNA we did not use the multiple-tube approach. Also, according to Morin et al. (2001) faecal DNA extracts with values higher than 201pg (0.201 ng) already guarantee high confidence (99% of certainty) and amplification precision of these extracts, being able to present allelic dropout in only 5.2% of samples. Due to the absence of null alleles and the observed heterozygosity higher than expected heterozygosity, we believe that genotyping error due to allelic dropout (genotype a heterozygous as a homozygous) did not occur in the sample set. The low concentration of DNA in our samples could also be a result of the storage time of the samples. For logistical reasons, we had to store samples from Serra do Estrago for one year, and samples from Serra do Pinheiro for nine months before the DNA extractions. The type and time of storage of faecal samples are thought to influence the success of DNA extraction (Piggott & Taylor 2003). There is no consensus on the best technique to be used, because often the type of storage will influence the choice of DNA extraction technique (Waits & Paetkau 2005, Vallet et al. 2007). Other factors also need to be taken into account in order to obtain a good sample, such as the type of diet of the species and the site where it was collected (Broquet et al. 2007). Herbivorous animals present secondary metabolites that may inhibit DNA amplification (Broquet et al. 2007, Vallet et al. 2007). Good quality DNA samples (from blood and tissue) are difficult to collect for species that are threatened, elusive, rare, occupy inaccessible areas or occur at low densities (Salgado-Lynn et al. 2016). Thus, testing and using samples collected in a non-invasive manner became a relevant alternative for conservation genetics studies (Di Fiore 2003, Broquet et al. 2007, Salgado-Lynn et al. 2016). The microsatellite markers transferred to *Sapajus libidinosus* can be widely used in different fields in the future such as population genetics, molecular ecology, population structure, gene flow and landscape connectivity studies. Thus, this validation of such molecular tools will contribute to the development of effective conservation strategies for capuchin monkeys and other primates.

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REFERENCES

BENCHIMOL M & PERES CA. 2013. Anthropogenic modulators of species–area relationships in Neotropical primates: a continental-scale analysis of fragmented forest landscapes. Divers Distrib 19: 1339-1352.

BOTSTEIN D, WHITE RL, SKOLNICK M & DAVIS RW. 1980. Construction of a genetic linkage map in man using restriction fragment length polymorphisms. Am J Hum Gen 32: 314-331.

BRADLEY BJ, STILLER M, DORAN-SHEEHY DM, HARRIS T, CHAPMAN CA, VIGILANT L & POINAR H. 2007. Plant DNA sequences from feces: potential means for assessing diets of wild primates. Am J Primatol 69: 699-705.

BROQUET T, MÉNARD N & PETIT E. 2007. Noninvasive population genetics: a review of sample source, diet, fragment length and microsatellite motif effects on amplification success and genotyping error rates. Conserv Gen 8: 249-260.

BUZATTI RSO, CHICATA FSL & LOVATO MB. 2016. Transferability of microsatellite markers across six *Dalbergia* (Fabaceae) species and their characterization for *Dalbergia miscolobium.* Biochem Syst Ecol 69: 161-165.

CHAMBERS KE, REICHARD UH, MÖLLER A, NOWAK K & VIGILANT L. 2004. Cross-species amplification of human microsatellite markers using non-invasive samples from white-handed gibbons (*Hylobates lar*). Am J Primatol 64: 19-27.

CORNUET JM AND LUIKARTT G. 1996. Description and power analysis of two tests for detecting recent population bottlenecks from allele frequency data. Genetics 144: 2001-2014.

DI FIORE A. 2003. Molecular genetic approaches to the study of primate behaviour, social organization, and reproduction. Am J Phys Anthropol 122: 62-99.

DI FIORE A, LINK A, SCHMITT CA & SPEHAR SN. 2009. Dispersal patterns in sympatric woolly and spider monkeys: integrating molecular and observational data. Behaviour 146: 437-470.

ESCOBAR-PÁRAMO P. 2000. Microsatellite primers for the wild brown capuchin monkey *Cebus apella.* Mol Ecol 9: 107-118.

EXCOFFIER L & LISCHER HEL. 2010. Arlequin suite ver 3.5: A new series of programs to perform population genetics analyses under Linux and Windows. Mol Ecol Resour. 10: 564-567.

FERREIRA RG, JERUSALINSKY L, SILVA TCF, FIALHO MS, ROQUE AA, FERNANDES A & ARRUDA F. 2009. On the occurrence of *Cebus flavius* (Schreber 1774) in the Caatinga, and the use of semi-arid environments by *Cebus* species in the Brazilian state of Rio Grande do Norte. Primates 50: 357-362.

FIALHO MS, VALENÇA-MONTENEGRO MM, BEZERRA BM, MARTINS AB & LAROQUE PO. 2015. Avaliação do Risco de Extinção de *Sapajus libidinosus* (Spix, 1823) no Brasil. Processo de avaliação do risco de extinção da fauna brasileira. ICMBio. http://www.icmbio.gov.br/portal/biodiversidade/fauna-brasileira/estado-de-conservacao/7274-mamiferos-sapajus-libidinosus-macaco-prego.htmll. Oficina de Avaliação do Estado de Conservação de Primatas Brasileiros. Accessed 15 May 2018.

GERLOFF U, HARTUNG B, FRUTH B, HOHMANN G & TAUTZ D. 1999. Intracommunity relationships, dispersal pattern and paternity success in a wild living community of Bonobos (*Pan paniscus*) determined from DNA analysis of faecal samples. Proc Roy Soc Lon, B: Biol Sci 266: 1189 -1195.

GOUDET J. 2002. Fstat, a Program to Estimate and Test Gene Diversities and Fixation Indices, Version 2.9.3.2. Available https://www2.unil.ch/popgen/softwares/fstat.htm

GROVES C. 2016. Primate (Taxonomy) The International Encyclopedia of Primatology. Edited by Agustín Fuentes. Australia: Australian National University.

GUO X & ELSTON RC. 1999. Linkage Information Content of Polymorphic Genetic Markers. Hum Hered 49: 112-118.

HOOGLAND JL, TROTT R & KELLER SR. 2019. Polyandry and polygyny in a social rodent: an integrative perspective based on social organization, copulations, and genetics. Frontiers in Ecology and Evolution 7: 1-15.

IUCN 2019. The IUCN Red List of Threatened Species. Version 2019-1. <https://www.iucnredlist.org>

KALINOWSKI ST, TAPER ML & MARSHALL TC. 2007. Revising how the computer program CERVUS accommodates genotyping error increases success in paternity assignment. Mol Ecol 16: 1099-1106.

KEARSE M ET AL. 2012. Geneious Basic: an integrated and extendable desktop software platform for the organization and analysis of sequence data. Bioinformatics 28: 1647-1649.

KOHN MH, YORK EC, KAMRADT DA, HAUGHT G, SAUVAJOT RM & WAYNE RK. 1999. Estimating population size by genotyping faeces. Proc Roy Soc Lon, B: Biol Sci 266: 657-663.

LEÓN-ORTEGA M & GONZÁLEZ-WANGÜEMERT M. 2015. Characterization of 10 new tetranucleotide microsatellite markers for the European eagle owl, *Bubo bubo*: Useful tools for conservation strategies. Biochem Syts Ecol 63: 109-112.

LI P, YANG C, ZHANG X, LI J, YI Y & YUE B. 2014. Development of eighteen tetranucleotide microsatellite markers in Tibetan Macaque (*Macaca thibetana*) and genetic diversity analysis of captive population. Biochem Syst Ecol 57: 293-296.

LIMA MGM ET AL. 2018. A phylogenomic perspective on the robust capuchin monkey (*Sapajus*) radiation: First evidence for extensive population admixture across South America. Mol Phyl Evol 124: 137-150.

LIU ZJ, REN BP, HAO YL, ZHANG HR, WEI FW & LI M. 2008. Identification of 13 human microsatellite markers via cross-species amplification of faecal samples from *Rhinopithecus bieti*. Int J Primatol 29: 265-272.

LIU F, MCSHEA WJ & LI D. 2017. Correlating habitat suitability with landscape connectivity: A case study of Sichuan golden monkey in China. Ecol Model 353: 37-46.

LYNCH-ALFARO JW, SILVA JR JS & RYLANDS AB. 2012. How different are robust and gracile capuchin monkeys? An argument for the use of *Sapajus* and *Cebus*. Am J Primatol 74: 273-286.

LYNCH-ALFARO J, IZAR P, & FERREIRA RG. 2014. Capuchin monkey research priorities and urgent issues. Am J Primatol 76: 705-720.

MORAES BLC, SOUTO AS & SCHIEL N. 2014. Adaptability in stone tool use by wild capuchin monkeys (*Sapajus libidinosus*). Am J Primatol 76: 967-977.

MORIN PA, CHAMBERS KE, BOESCH C & VIGILANT L. 2001. Quantitative polymerase chain reaction analysis of DNA from noninvasive samples for accurate microsatellite genotyping of wild chimpanzees (*Pan troglodytes verus*). Mol Ecol 10: 1835-1844.

MUNIZ L & VIGILANT L. 2008. Isolation and characterization of microsatellite markers in the white-faced capuchin monkey (*Cebus capucinus*) and cross-species amplification in other New World monkeys. Mol Ecol Resour 8: 402-405.

OLIVEIRA EJ, PÁDUA JG, ZUCCHI MI, VENCOVSKY R & VIEIRA MLC. 2006. Origin, evolution and genome distribution of microsatellites. Gen Mol Biol 29: 294-307.

PIGGOTT MP & TAYLOR AC. 2003. Remote collection of animal DNA and its applications in conservation management and understanding the population biology of rare and cryptic species. Wild Res 30: 1-13.

PRADO DE. 2003. As caatingas da América do Sul. In: Leal IR, Tabarelli M & Sila JMC (Eds), Ecologia e conservação da Caatinga, Recife: Universitária da UFPE.

RATTER JA, RIBEIRO JF & BRIDGEWATER S. 1997. The Brazilian Cerrado Vegetation and Threats to its Biodiversity. Ann Bot 80: 223-230.

RICE WR. 1989. Analyzing tables of statistical tests. Evolution 43: 223-225.

RUIZ-LOPEZ MJ, BARELLI C, ROVERO F, HODGES K, ROOS C, PETERMAN WE & TING DN. 2016. A novel landscape genetic approach demonstrates the effects of human disturbance on the Udzungwa red colobus monkey (*Procolobus gordonorum*). Heredity 116: 167-176.

RYLANDS AB & KIERULFF MCM. 2015. *Sapajus libidinosus*. *The IUCN Red List of Threatened Species* 2015: e.T136346A70613080. http://dx.doi.org/10.2305/IUCN.UK.2015-1.RLTS.T136346A70613080.en. Accessed 05 May 2017.

SALGADO-LYNN M, SECHI P, CHIKHI L & GOOSSENS B. 2016. Primate conservation genetics at the dawn of conservation genomics. In: Wich SA & Marshall AJ (Eds), An Introduction to Primate Conservation, Oxford University Press, p. 53-77.

SILVA JJS. 2001. Especiação nos macacos-prego e caiararas, gênero *Cebus* Erxeleben, 1777 (Primates, Cebidae). MSc Dissertation: Universidade Federal do Rio de Janeiro. (Unpublished).

SRBEK-ARAUJO AC, HAAG T, CHIARELLO GA, SALZANO FM & EIZIRIK E. 2018. Worrisome isolation: noninvasive genetic analyses shed light on the critical status of a remnant jaguar population. J Mammal 99: 397-407.

STEVANOVIC J, STANIMIROVIC Z, DIMITRIJEVIC V & MALETIC M. 2010. Evaluation of 11 microsatellite loci for their use in paternity testing in Yugoslav Pied cattle (YU Simmental cattle). Czech J Anim Scie 55: 221-226.

STORFER A, PATTON A & FRAIK AK. 2018. Navigating the interface between landscape genetics and landscape genomics. Front Gen 9: 1-14.

TIAN C, GUO W, LIANG X, SUN L, LVL, ZHAO C, SONG Y & HE S. 2017. Identification of species-specific microsatellite markers in three *Siniperca* species by RNA-Seq. Biochem Syts Ecol 70: 126-131.

TOKUDA M. 2012. Dispersão de macacos-prego (*Sapajus nigritus*) do Parque Estadual Carlos Botelho, São Paulo. PhD, University of São Paulo.

TOKUDA M, MARTINS MM & IZAR P. 2014. Transferability of microsatellites for studies on the social behaviour of the tufted capuchin monkey (genus *Sapajus*). Gen Mol Res 13: 9910-9914.

VALLET D, PETIT EJ, GATTI S, LEVRÉRO F & MÉNARD N. 2007. A new 2CTAB/PCI method improves DNA amplification success from faeces of Mediterranean (*Barbary macaques*) and tropical (lowland gorillas) primates. Cons Gen 9: 677-680.

VAN OOSTERHOUT C, HUTCHINSON WF, WILLS DPM & PETER S 2004. MICRO-CHECKER: software for identifying and correcting genotyping errors in microsatellite data. Mol Ecol Notes 4: 535-538.

VERDERANE MP. 2010. Socioecologia em macacos-prego (*Cebus libidinosus*) em área de ecótono Cerrado em Caatinga. PhD thesis, Universidade de São Paulo, Brasil.

WAITS LP, LUIKART G & TABERLET P. 2001. Estimating the probability of identify among genotypes in natural populations: cautions and guidelines. Mol Ecol 10: 249-256.

WAITS L & PAETKAU D. 2005. Noninvasive genetic sampling tools for wildlife biologists: a review of applications and recommendations for accurate data collection. J Wild Manag 69: 1419-1433.

WANG B, WANG ZL, TIAN JD, CUI ZW & LU JI-QI. 2015. Establishment of a microsatellite set for noninvasive paternity testing in free-ranging *Macaca mulatta tcheliensis* in Mount Taihangshan area, Jiyuan, China. Zool Stud 54: 8. https://doi.org/10.1186/s40555-014-0100-9.

YEWERS MSTC, STUART-FOX D & MCLEAN CA. 2019 Space use and genetic structure do not maintain color polymorphism in a species with alternative behavioral strategies. Ecol Evol 9: 295-306.

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