Characterization of Satellite DNAs in Squirrel Monkeys genus *Saimiri* (Cebidae, Platyrhini)

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The genus *Saimiri* is a decades-long taxonomic and phylogenetic puzzle to which cytogenetics has contributed crucial data. All *Saimiri* species apparently have a diploid number of $2n = 44$ but vary in the number of chromosome arms. Repetitive sequences such as satellite DNAs are potentially informative cytogenetic markers because they display high evolutionary rates. Our goal is to increase the pertinent karyological data by more fully characterizing satellite DNA sequences in the *Saimiri* genus. We were able to identify two abundant satellite DNAs, alpha (~340 bp) and CapA (~1,500 bp), from short-read clustering of sequencing datasets from *S. boliviensis*. The alpha sequences comprise about 1% and the CapA 2.2% of the *S. boliviensis* genome. We also mapped both satellite DNAs in *S. boliviensis, S. sciureus, S. vanzolinii*, and *S. ustus*. The alpha has high interspecific repeat homogeneity and was mapped to the centromeres of all analyzed species. CapA is associated with non-pericentromeric heterochromatin and its distribution varies among *Saimiri* species. We conclude that CapA genomic distribution and its pervasiveness across Platyrhini makes it an attractive cytogenetic marker for *Saimiri* and other New World monkeys.

Squirrel monkeys of the genus *Saimiri* (Cebidae, Platyrhini) are medium sized neotropical primates inhabiting forest environments of South America. They range from about 10°N to 17°S including the Amazon basin, the Guianas, and coastal zones of Central America1–4. As for many other New World monkey (NWM) taxa the phylogenetic relationships within the genus *Saimiri* are still debated1. Even the number of species is uncertain, historically ranging from one to 16 distinguished species5–11. In a recent molecular report on mitochondrial D-Loop and *cyt b* sequences, Alfaro et al.3 presented a provisional taxonomy of seven *Saimiri* species and various subspecies: (1) *S. boliviensis*, (2) *S. cassiquiarensis* (*S. c. cassiquiarensis, S. c. albigena, S. c. macrodon A, S. c. macrodon B*, and *S. c. macrodon C*), (3) *S. collinsi*, (4) *S. oerstedii* (*S. o. oerstedii and S. o. citronellus*), (5) *S. sciureus*, (6) *S. ustus* (*A, B, and C lineages*), and (7) *S. vanzolinii*.

Cytogenetic studies consistently showed that all *Saimiri* have a diploid number of $2n = 44$, but can differ in fundamental numbers (FN, the number of chromosome arms) which range from 74 to 7812. Differences in FN in *Saimiri* have traditionally been expressed as the number of acrocentric chromosomes, with five (FN = 78) to seven pairs (FN = 74). FN variation was thought to correlate with geographic distribution and taxonomy. According to Jones et al.13 individuals from Costa Rica, Panama and Iquitos – Peru had five acrocentric pairs (FN = 78), those originating from Leticia – Colombia had six pairs (FN = 76) and specimens from Georgetown – Guiana had seven pairs of acrocentric chromosomes (FN = 74). Differences in FN in *Saimiri* were previously thought to be the result of pericentric inversions and reciprocal translocations14–16 but recently Chiatante et al.12, using high-resolution BAC-FISH analysis, showed that centromere repositioning explains differences in FN in *Saimiri*. These authors, however, did not address the taxonomic issue within the genus.

A class of markers that can be used to study karyotype evolution and address taxonomic issues is satellite DNAs (satDNAs). These sequences consist of tandem repeats organized in large arrays (up to Mb size) typically associated with chromosome landmarks such as centromeres, telomeres, and heterochromeric regions [e.g.17–19].
Several satDNAs show a concerted mode of evolution, in which new mutations are homogenized within satDNA arrays in a genome and differentially fixed in reproductively isolated populations [reviewed in\(^{20–22}\)]. Mechanisms such as gene conversion and unequal crossing-over are involved in the evolutionary process known as molecular drive, responsible for the concerted evolution of satDNAs\(^{21}\). The rapid concerted evolution of satDNAs can result in high intraspecific sequence homogeneity and interspecific differences, making satDNAs potential taxonomic markers and, in some cases, allowing their use for phylogenetic inferences\(^{9,23}\). SatDNAs have been used as cytogenetic markers facilitating species identification in many taxa, including primates\(^{24}\), frogs\(^{25}\), fish\(^{26}\) and plants\(^{27}\).

The alpha is the most studied satDNA in primates. It has a centromeric location and its monomer length in Old World primates is \(~170\) bp. Most NWMs (Platyrrhini) have a derived alpha with \(~340\) bp but species of the Pitheciidae family have a monomer of \(~550\) bp composed by four \(~170\) bp subunits with the third one incomplete\(^{17,28,29}\). Alpha satDNA is often highly divergent among species and also among chromosomes of the same species\(^{18,28}\). CapA is a satDNA present only in NWMs, with \(~1,500\) bp monomer length and was found in the three Platyrrhini families, with different chromosome localization and abundance varying from less than 1% up to 5%\(^{17,30,31}\).

The repetitive DNA fraction of the Saimiri genomes, including their satDNAs, are largely unexplored. In this work, we employed bioinformatic and cytogenetic tools to characterize the satDNAs of Saimiri. We characterized the two most abundant satDNAs of the genus and used these sequences to analyze the karyotypes of several individuals. Alpha and CapA comprise \(~1\)% and 2.2% of the S. boliviensis genome, respectively. The alpha satDNA has \(~340\) bp, a centromeric location and high interspecific monomer homogeneity, while CapA has \(~1,500\) bp and is associated with constitutive heterochromatin. This satDNA was mainly located in distal regions of the short arms and in the interstitial heterochromatin of some chromosomes, showing different chromosome localization among Saimiri species. Novel markers may help to clarify the taxonomic and phylogenetic relationships among Saimiri taxa.

### Results

#### Chromosome banding.

The 12 Saimiri individuals analyzed presented the expected diploid number of \(2n = 44\), but their fundamental numbers (FNs) varied due to the presence of different numbers of acrocentric chromosomes, which ranged from 10 to 14 (five to seven pairs) (Table 1). The karyotypes were arranged according to Stanyon et al.\(^{32}\). GTG-banding allowed the identification of all chromosomes.

| Species          | Specimen | Sex | Origin                        | FN  | Pair 5 | Pair 15 |
|------------------|----------|-----|-------------------------------|-----|--------|--------|
| S. boliviensis   | SBO1     | Male| Unknown                       | 76  | SM     | A      |
| S. sciureus      | SSC782   | Male| Presidente Figueiredo – Amazonas | 74  | A      | A      |
| S. sciureus      | SSC770   | Female| Santarém – Pará               | 74  | A      | A      |
| S. sciureus      | SSC2     | Male| Unknown                       | 74  | A      | A      |
| S. vanzolinii    | SYA321   | Female| Lake Mamirauá, Tefé - Amazonas | 76  | SM     | A      |
| S. vanzolinii    | SYA322   | Male| Lake Mamirauá, Tefé - Amazonas | 76  | SM     | A      |
| S. ustus         | SUS739   | Male| Hydroelectric plant, Samuel - Rondônia | 78   | SM     | SM     |
| S. ustus         | SUS740   | Female| Hydroelectric plant, Samuel - Rondônia | 78   | SM     | SM     |
| S. ustus         | SUS742   | Female| Hydroelectric plant, Samuel - Rondônia | 78   | SM     | SM     |
| S. ustus         | SUS746   | Female| Hydroelectric plant, Samuel - Rondônia | 78   | SM     | SM     |
| S. ustus         | SUS747   | Female| Hydroelectric plant, Samuel - Rondônia | 78   | SM     | SM     |
| S. ustus         | SUS786   | Female| Hydroelectric plant, Samuel - Rondônia | 78   | SM     | SM     |

Table 1. Identification, sex, collection site, fundamental number and morphology of chromosome pairs 5 and 15 of the analyzed specimens. SBO - Saimiri boliviensis; SSC - Saimiri sciureus; SYA - Saimiri vanzolinii; SUS - Saimiri ustus; FN – fundamental number; SM - submetacentric; A - acrocentric.
bands were detected in pairs 5 and 15 of all S. ustus analyzed. In SSC782 and SBO1, a CBG band was detected only in one homologue of pair 14, in the proximal and distal regions of the short arm.

**Satellite DNAs identification and chromosome mapping.** After careful analysis of RepeatExplorer’s results, we identified two clusters that corresponded to potential satDNAs. Cluster 5 (CL5) comprises 22,193 reads (out of 2,230,692), representing ~1% of the S. boliviensis genome. RepeatExplorer includes partial assembly of reads into contigs and RepeatMasking of contigs using the RepBase metazoan library. This analysis indicated that CL5 corresponded to the centromeric repeat. Extending the search for CL5 sequences to the nr/nt GenBank database revealed that this cluster represents the well-known alpha satDNA, known to have a centromeric location in simian primates. Similarity searches of CL5 sequences against the S. boliviensis reference genome (accession GCA_000235385.1) revealed that these sequences are organized in tandem and that monomers are ~340 bp in length, which was confirmed by PCR (Supplementary Fig. S3a).

Fluorescent in situ hybridization (FISH) with the alpha satDNA in squirrel monkey chromosomes revealed its presence in the centromeric region of all chromosomes in S. boliviensis, S. sciureus, and S. vanzolinii (Fig. 2). In S. ustus it was absent from pair 12 and from one homologue of pair 7. Furthermore, the hybridization signal in chromosome 6 of SSC770, SSC 782 and SSC2 was much weaker when compared to the other species. In all Saimiri species, the alpha satDNA signal was more intense in acrocentric chromosomes and in the metacentric pair 19.

The second potential satDNA cluster, CL3, was initially found split into four clusters with a strongly connected component to each other (CL6, CL7, CL8 and CL12). These were merged using the RepeatExplorer cluster merger tool, thus recovering a larger CL3. This cluster comprises 49,193 reads, or ~2.2% of the genome, representing the second most abundant repeat family in the S. boliviensis genome (Supplementary Table S1). Analysis of this sequence in the S. boliviensis reference genome revealed tandem repeats with a ~1,500 bp monomer length confirmed by PCR (Supplementary Fig. S3b). Similarity searches on the nr/nt database from GenBank using partially

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**Figure 1.** (a) Representative Saimiri karyotype with FN = 78 and five acrocentric pairs, pairs 5 and 15 are submetacentric (SUS 739); the lower panels show the acrocentric pairs 5 and 15 (SSC 770, SSC 782, SSC2); submetacentric pair 5 and acrocentric 15 (SBO1, SVA 321 and SVA 322), submetacentric pairs 5 and 15 (SUS). (b) CBG-banded Saimiri karyotype with FN = 76 (SVA 321), and in the boxes below pairs 2, 5, 15 and 14 from S. sciureus (SSC), S. boliviensis (SBO), S. vanzolinii (SVA) and S. ustus (SUS). Chromosome pair 2 is highlighted in blue, pair 5 in green, pair 14 in gray and pair 15 in pink. Bar = 10 μm.
assembled contigs from the reads of CL3 as queries revealed that this sequence is homologous to a satDNA named CapA described in *Sapajus apella*\(^\text{17}\). We have previously obtained a probe for CapA when studying the origin and distribution of this satDNA in mammals\(^\text{31}\) and we used the same probe to analyze CapA distribution in the *Saimiri* genus. FISH with the CapA probe showed a distribution largely coincident with the heterochromatic regions revealed after CBG-banding in the four *Saimiri* species. Signals were mainly located in the distal regions of the short arms of submetacentric chromosomes and in the interstitial heterochromatin of some chromosome pairs (Fig. 3). There was a slight variation in CapA localization among *Saimiri* species. The difference of CapA localization between *S. boliviensis* and *S. vanzolinii*, that share the same FN = 76, was its presence in chromosome 14 of *S. boliviensis* and its absence in the same chromosome of *S. vanzolinii*. Besides *S. boliviensis*, only *S. sciureus* had CapA mapped to pair 14. Only in *S. sciureus* (FN = 74) CapA was not detected in pair 11 and its signal was much more intense in pair 2. CapA was not detected in pair 13 of *S. sciureus* and *S. ustus* (FN = 78). As observed with CBG bands, pairs 5 and 15 had distal CapA signals in their short arms only in their submetacentric form. Thus, *S. sciureus* did not show CapA in pairs 5 and 15; *S. boliviensis* and *S. vanzolinii* had the satDNA mapped in pair 5 and *S. ustus* in both pairs.

**Alpha satDNA sequence analysis.** Sequences of the alpha satDNA were obtained for all four *Saimiri* species studied herein from either the reference genome (*S. boliviensis*), from clones obtained by Kugou et al.\(^\text{33}\) for *S. sciureus*, and from cloning and Sanger sequencing performed in this study (*S. sciureus, S. vanzolinii* and *S. ustus*). These sequences were aligned and compared for intra and interspecies diversity, using neighbor-joining clustering and non-metric multidimensional scaling (NMDS) ordinations. Alpha sequences showed the same value (0.16 substitutions per site) for intra and interspecific diversity. Both the neighbor-joining tree and NMDS ordinations revealed that alpha sequences do not display species-specific clustering (Fig. 4). Neighbor-joining clustering suggested a divergent group of alpha sequences, but in a branch without support. Interestingly, NMDS
showed the same group of alpha sequences clearly distinct from the others, indicating a potential chromosome variant. The neighbor-joining and NMDS results were supported by the Maximum Likelihood method (Tamura 3-parameter model) analysis (Supplementary Fig. S4).

Discussion
We identified two abundant satDNAs in the genome of *Saimiri boliviensis*, alpha and CapA. We characterized both for repeat size, abundance, and chromosome localization in *S. boliviensis*, *S. sciureus* *S. vanzolinii* (SVA 322) and *S. ustus* (SUS 739). Bar = 10 μm.

Figure 3. FISH with CapA in the karyotypes of *S. boliviensis* (SBO1), *S. sciureus* (SSC 782 and SSC2), *S. vanzolinii* (SVA 322) and *S. ustus* (SUS 739). Bar = 10 μm.
The alpha satDNA centromeric localization was observed in all chromosomes of *S. boliviensis*, *S. sciureus* and *S. vanzolinii*. Moreover, it appears to be absent or undetectable by FISH (due to low repeat number or sequence divergence) from chromosomes 7 and 12 in *S. ustus* (Fig. 5). Variable FNs among squirrel monkey species is due to centromere repositioning in pairs 5 and 15 [12]. Our sample includes specimens with both morphologies of chromosomes 5 and 15 and the alpha satDNA was always detected at their centromeres, indicating that these evolutionary new centromeres (ENCs) are mature.

The alphoid satDNA family is known to be part of the centromere in simian primates (Catarrhini and Platyrrhini). In a phylogenetic analysis of primate alpha satDNA, Alkan et al. [18] reported that alpha satDNA repeats are evolutionarily distinct and heterogeneous between human, chimpanzee and macaques. Alpha DNA was shown to have a genus-specific chromosome localization in small apes from the *Nomascus* and *Hylobates* genera, which could be used as a cytogenetic marker [24].

**Figure 4.** (a) Neighbor-joining clustering of alpha sequences of *S. boliviensis* (SBO), *S. sciureus* (SSC), *S. vanzolinii* (SVA) and *S. ustus* (SUS) inferred by the neighbor-joining method with 1000 bootstrap replicates. Tree visualized in ITOL v4.3.3 (https://itol.embl.de/) [52]. (b) Non-metric multidimensional scaling (NMDS) of evolutionary divergence among the same alpha sequences used in the phylogenetic analyses. The scaling represents euclidean distances for two dimensions (stress: 0.1127148). The sequences present in the left group of ordinations are the same of the branch in bold of the phylogenetic tree. Colors indicate the sequence taxa (bottom).
Our sequence analysis of alpha repeats between *Saimiri* species revealed a high interspecific repeat homogeneity. The homogeneity found among alpha repeats of all analyzed species may be due to the recent *Saimiri* species diversification (1.4–0.8 million years ago–Mya) and/or to hybridization events. In fact, hybridization has been reported between *S. sciureus* and *S. ustus*34, between *S. boliviensis* and *S. sciureus*, and between *S. boliviensis* and *S. ustus*. Another possibility is that the alpha repeats have been conserved due to their important role in centromere function and maintenance. For example, Smalec et al.36 reported a conserved centromeric repeat (PM sat) among rodents of the genus *Peromyscus* and suggested that molecular drive was not the only agent in the evolution of this satDNA and that the homologous arrays may play a role in the chromosome evolution of the genus.

NMDS ordinations and neighbor-joining clustering clearly split *Saimiri* alpha repeats into two groups, one containing most sequences and the other containing only a few sequences but representing all four species. This could be an indication that monomers are evolving divergently among the chromosomes. In humans, alpha is known to have chromosome-specific sequences and in *Callitrichus jacchus* alpha repeats are divided into seven clusters that showed different hybridization patterns among chromosomes.

The second satDNA analyzed, CapA, has ~1,500 bp monomers, comprises ~2.2% of the *S. boliviensis* genome and is associated with constitutive heterochromatin. It is the second most abundant repetition in *S. boliviensis*, only surpassed by Alu transposable elements (Short Interspersed Nuclear Elements – SINEs). CapA was first identified after digestion of *Sapajus apella* (previously classified as *Cebus apella*) genomic DNA with restriction enzymes, and was shown to be ~1,500 bp long and to comprise about 5% of the genome. In a recent study, we provided evidence that the CapA satDNA is homologous to an intronic sequence of the NOS1AP gene (*Homo sapiens* nitric oxide synthase 1 adaptor protein), and likely originated from this single-copy sequence through duplication and unequal crossing-over. We also showed that CapA is present in representatives of the three Platyrrhini families (Cebidae, Atelidae and Pitheciidae; except in Callitrichines and in the *Callitrichus* genus) with different genome abundance and chromosome localization, always associated with constitutive heterochromatin.

CapA repeats in *Saimiri* chromosomes are mostly enriched in the distal heterochromatin of submetacentric chromosomes, but the overall distribution and abundance differed among species (Fig. 5).

Interestingly, CapA was mapped in some chromosomes involved in rearrangements. For instance, CapA distribution in *Saimiri* chromosomes 5 and 15 was coincident with the CBG bands: they were absent in the acrocentric forms and present in the distal heterochromatin of the short arm in the submetacentric variants of these chromosomes. This variation may be related to the morphology of these chromosomes and perhaps with the process of ENCs formation in *Saimiri*. According to the putative ancestral Platyrrhini karyotype, the ancestral morphology of chromosome 5 and 15 is submetacentric and acrocentric, respectively32,36. Taking this into account, chromosome 5 would have lost CapA sequences when the chromosome became acrocentric and the new centromere occupied this region. Conversely, in chromosome 15, the CapA satDNA would have colonized the old centromere region after the chromosome became submetacentric.

SBO1 was previously analyzed by Chiatante et al.12, who reported pair 14 as heteromorphic, with isoforms A and B differing by a paracentric inversion and two heterochromatic blocks, one distal and one in the proximal region of the short arm. This same polymorphism was also described in several specimens of squirrel monkeys, especially in those from Bolivia, in which heterozygotes for pair 14 were more common than homozygotes. Chiatante et al.12 suggested that an inversion in the isoforn B may have carried some telomeric DNA sequences to the interstitial region of the short arm, explaining the heterochromatic blocks. Three of our specimens (SBO1, SSC 782 and SSC2) presented the isoforn B of chromosome 14, and in all of them CapA was located in the interstitial heterochromatin and distal region. In SSC 782, CapA was also detected in the distal region of the short arm of isoform A. This indicates that CapA could have been involved in the generation of this paracentric inversion, instead of the suggested telomeric sequences12.
Chromosome painting with human probes revealed that Saimiri 2q is formed by homologues of human chromosomes 14 and 15 (HSA 14 and 15) and their signals are intercalated due to inversions. CapA was mapped on 2q in a region that may correspond to the breakpoint of one of these inversions. CapA was also observed in Saimiri 2p, which is homologous to HSA 9. In Sapajus apella HSA 9 corresponds to pair 19, which has an interstitial heterochromatic block where CapA was observed and corresponds to an inversion. Pair 7 of Saimiri is formed by the association of HSA 15/HSA 2, and presents CapA in the short arm of most specimens analyzed (Fig. 5), although seemingly not at the association region.

Although our data do not allow to pinpoint CapA to chromosome rearrangements, they indicate that a detailed analysis of these regions could provide some new interesting data.

CapA distribution differed more among Saimiri species than CBG bands. Even individuals of different species with the same karyotype and FN differed in their CapA localization, as for instance, S. boliviensis and S. vanzolini, both with FN = 76 (Fig. 3). The specimens identified as S. sciureus (FN = 74) did not show CapA in pair 11 and the satDNA was more abundant in pair 2 when compared with the other species (Fig. 3). The specimen SSC 770 identified as S. sciureus has a similar karyotype and CapA distribution to SSC 782, however its collection site suggests that it may actually be a S. collinsi according to Alfaro et al. No karyotype has been described for S. collinsi or for samples collected within its geographic distribution, preventing us from further conclusions.

The variable chromosomal localization of CapA among the Saimiri species analyzed herein suggests that, combined with FN, this sequence may be used as a valuable tool in taxonomic identification. Hybridization in Saimiri was obtained from cultured cells according to Stanyon and Galleni. GTG- and CBG-banding were performed in order to further validate CapA as a marker, more geotagged specimens need to be analyzed. The study of species not yet karyotyped will also help clarify Saimiri’s taxonomic puzzle and inform its systematics.

Our results indicate that CapA is a promising cytogenetic marker and that it could be useful for taxonomic, phylogenetic and conservation studies of Saimiri. In addition, the fact that CapA is present across Platyrrhini further extends its utility as a marker for chromosome and genome evolution studies in NWMs. The availability of new markers is especially important in the face of threats of extinction to an alarming number of NWM species due to rapid habitat loss.

Materials and Methods

Biological samples and chromosome banding. Chromosome spreads and genomic DNAs were obtained from fibroblast cultures of 12 squirrel monkeys: one S. boliviensis, three S. sciureus, two S. vanzolini, and six S. ustus. We used previously established cell lines and the available details for the samples are provided in Table 1. The work did not involve the direct use of animals, so ethical permission was not required. Genomic DNAs were purified with the Wizard Genomic DNA Purification Kit (Promega) and chromosome spreads were obtained from cultured cells according to Stanyon and Galleni. GTG- and CBG-banding were performed according to Seabright and Sumner, respectively. The samples from S. boliviensis (SBO1) were previously analyzed in Chiatante et al. and Capozzi et al. The S. sciureus (SCC2) sample was previously reported in Chiatante et al. The two S. vanzolinii (SVA 321 and SVA 322) samples were previously reported in Yonenaga-Yassuda and Chu.

Satellite DNAs identification. The RepeatExplorer pipeline was used to identify satDNAs based on all to all similarity comparison of the Illumina reads of S. boliviensis (NCBI SRA access: SRR317821). A total of 2,230,692 Illumina reads (~100 bp long) comprising 7% of the estimated S. boliviensis genome were randomly sampled and used in this analysis. The results are represented as graph-based clusters of similar reads and the shape of the clusters is indicative of the nature of the different repeat families (e.g. globula and ring-like structures suggest tandemly organized repeats). The number of reads within the clusters (out of the total used in the analysis) indicates the abundance of that cluster in the genome. The clustering process tends to split large repeats into several clusters, but the pipeline has a separate re-clustering tool for a user-aided merging of the clusters. The reads that make up each cluster are partially assembled into contigs that can be used for repeat annotation. The minimum overlapping lengths used for clustering and assembly were 55 and 40 bp, respectively. Sequences in clusters with globular/ring-like structure were analyzed in detail through similarity searches against the S. boliviensis reference genome (accession GCA_000235385.1) using the BLASTn tool with default parameters. Additionally, satDNA clusters were annotated through similarity searches against the whole non-redundant nucleotide collection in GenBank.

PCR amplification, cloning and sequencing of satellite DNAs. Isolation of alpha satDNA was performed through polymerase chain reaction (PCR) of Saimiri genomic DNAs using the following specific primer set: alpha-F (ACAGGGAAATATCTGCTTCTAAATC) and alpha-R (GCTTACTGCTGTTTCTTCCATATG). The thermocycling conditions were as follows: 95°C—3 min, 35 cycles: 95°C—30 sec, 60°C—30 sec, 72°C—1 min;
final elongation: 72 °C—3 min. The repeat monomers obtained by PCR were cloned into pGEM-T Easy vector plasmids (Promega) and used to transform E. coli strain XL1-BLUE (Phoneutria) through electroporation. Recombinant colonies were capillary sequenced with the ABI3130 platform (Applied Biosystems) and are available in GenBank under accession numbers MK879580-MK879592. We obtained three sequenced clones from S. sciureus, five from S. vanzolinii and five from S. ustus. These sequenced clones were used in molecular analyses and as FISH probes. CapA amplification was performed using genomic DNAs from S. boliviensis and S. vanzolinii and the primer set CapA-F (ACTTCTCCTGACCTGTCTT) and CapA-R (GGGCTGATGCTTAATGTAGCA). CapA isolation and cloning were previously performed in Valeri et al.31 using human DNA and the same primer set described. The sequenced product is deposited in GenBank under the accession number MG264524 and was used as a FISH probe in this study.

Fluorescent in situ hybridization (FISH). FISH was performed using alpha and CapA sequences as probes on metaphase spreads of the four Saimiri species. SatDNA probes were prepared from pGEM-T Easy cloned sequences and labeled by nick translation with biotin-16-dUTP or digoxigenin-11-dUTP (Nick Translation mix, Roche Applied Science). Chromosomes were denatured in 70% formamide/2xSSC (saline-sodium citrate buffer) at 75% for 105 sec. The hybridization mix consisted of 100 ng of labeled probe in 50% formamide/2xSSC and was denatured for 10 min at 98 °C and added to the chromosome spreads. Hybridization was carried at 37 °C for 16–20 hours. Post-hybridization washes consisted of three baths of 2xSSC at 45 °C for 5 min each. Immunodetection was performed with neutravidin+rhodamine or avidin+FITC conjugates (Roche Applied Science) and the slides were mounted with DAPI 1:500 in Slowfade (Life Technologies). Chromosome identification was based on the Q-banding pattern produced after DAPI staining. The analyses were performed under a Zeiss Axiosimager 2 epifluorescence microscope equipped with a CCD camera and image acquisition was performed with the AxioVision software (Carl Zeiss Microlmaging, Jena, Germany).

Alpha satDNA sequence analyses. In order to test if satDNA sequences of a species showed signs of concerted evolution we aligned the satDNA sequence monomers with MUSCLE32 and performed clustering using the neighbor-joining method with 1000 bootstrap replicates in MEGA X. Alpha sequences intra and interspecific diversities were calculated using the Maximum Composite Likelihood model in MEGA X. In addition, we computed pairwise distances between all sequences using a Maximum Composite Likelihood model in MEGA X and used these data to perform a NMDS analysis. The dataset used for these analyses was mainly composed of alpha monomeric sequences retrieved from GenBank (https://www.ncbi.nlm.nih.gov/genbank/). Thus, we performed BLASTn using the alpha consensus sequence as query against the S. boliviensis reference genome (accession GCA_000235385.1) and S. sciureus alpha clones (accession LC075928-LC075953) obtained by Kugou et al.33. Because GenBank lacks data from S. vanzolinii and S. ustus, we added sequences of these species obtained herein by cloning and Sanger sequencing. The total of alpha monomeric sequences used was 120, composed by 67 from S. boliviensis, 43 from S. sciureus, five from S. vanzolinii and five from S. ustus. The resulting neighbor-joining tree was visualized in iTOL v4.3.3 (https://itol.embl.de/)34. NMDS ordinations were generated with the R package vegan35, representing the divergence between sequences values in Euclidian distances for two dimensions. RStudio v1.1.46354 was used to conduct the NMDS analyses and to plot the ordinations.

Data availability The datasets generated during and/or analyzed in the current study are available in the GenBank repository (https://www.ncbi.nlm.nih.gov/genbank/) and in NCBI trace and short-read archive (https://trace.ncbi.nlm.nih.gov/Traces/sra/sra.cgi?). All accession numbers are provided in the Methods section.

Received: 3 December 2019; Accepted: 15 April 2020; Published online: 08 May 2020

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Author contributions
M.P.V. and G.B.D. carried out bioinformatic, cytogenetic and molecular analyses, participated in data analyses, designed the study and drafted the manuscript; M.P.V. prepared all figures; R.S. carried out cytogenetic analyses, participated in data analyses and helped drafting the manuscript; C.N.M., Y.Y. and R.S. obtained the materials for molecular and cytological analyses; G.B.D., G.C.S.K. and M.S. conceived and coordinated the study, and helped drafting the manuscript. All authors gave final approval for publication.

Competing interests
The authors declare no competing interests.

Additional information
Supplementary information is available for this paper at https://doi.org/10.1038/s41598-020-64620-1.

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