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FILOGEOGRAFIA DO MURIQUI DO SUL, *Brachyteles arachnoides* (PRIMATES, ATELIDAE)

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E vamos ao que realmente interessa para você que está lendo essa dissertação, a seguir cenas emocionantes do meu trabalho de mestrado.

Resumo

A espécie *Brachyteles arachnoides*, popularmente conhecido como muriqui do sul, é considerado o maior primata da América Latina e listado atualmente como estando ameaçado de extinção, apesar disso, até o momento, não existem estudos moleculares de genética de população a seu respeito. Aqui nós analisamos o padrão filogeográfico, diversidade genética e história demográfica da espécie utilizando sequências de parte da Região Controle do mtDNA e 14 loci de microssatélites. O mtDNA mostrou uma alta diversidade genética e nenhum indício claro de estruturação geográfica. Além disso, mostrou-se que houve uma expansão populacional há cerca de 15 mil anos atrás e não há nenhuma evidência concreta de declínio populacional. Assim como nas análises com mtDNA, os microssatélites também mostraram falta de estruturação geográfica, ausência de grupos definidos e nenhuma evidência de declínio populacional recente. A ausência de estruturação geográfica era esperada, principalmente para os dados de mtDNA, visto que as fêmeas são conhecidas por migrarem do seu grupo natal, além disso os grupos sociais de muriqui são formados por machos e fêmeas sem uma hierarquia aparente. Por outro lado, como os loci de microssatélite usualmente respondem rapidamente à fragmentação recente, seria esperado que eles mostrassem alguma evidência de estruturação causada pela recente fragmentação na Mata Atlântica, o que não foi observado. Resultados semelhantes também foram encontrados com o muriqui do norte (*Brachyteles hypoxanthus*) que está criticamente ameaçado e ocorre em áreas mais fragmentadas.

Abstract

The species *Brachyteles arachnoides*, popularly known as southern muriqui, is considered the largest neotropical primate and currently listed as endangered, nevertheless no molecular population genetics study has been done so far in the southern muriqui. Here we analyze the phylogeography pattern, genetic diversity and demographic history of this species using sequences from part of the mtDNA Control Region and 14 microsatellites. The mtDNA showed a high genetic diversity and no clear evidence of geographical structuring. Moreover we showed that there was a population expansion around 15 thousand years ago and there is no concrete evidence of population decline. As well as in analysis with mtDNA, the microsatellites also showed no geographical structure, groups were not clearly defined and recent population decline was not evident. The lack of geographical structure was expected, mainly for mtDNA data, since females are known to disperse from their natal group, moreover the social groups are formed by male and females without an apparent hierarchy. For the other hand, as the microsatellites usually respond more rapidly to more recent fragmentation it would be expected they showed some evidence of structure caused by the recent fragmentation of Atlantic Forest, which was not observed. Similar results were also observed with the northern muriqui (*Brachyteles hypoxanthus*) that is critically endangered and occurs in more fragmented areas.

1. Introdução Geral

O gênero *Brachyteles* pertence à família Atelidae. No passado, o gênero era considerado monoespecífico, sendo a única espécie reconhecida chamada *Brachyteles arachnoides*, distribuída do sul da Bahia até o norte do Paraná ao longo da Floresta Atlântica. Em 1944, Vieira sugeriu a subdivisão da espécie *B. arachnoides* em duas subespécies, a partir de algumas diferenças morfológicas tais como a coloração da face e a presença ou ausência de polegar vestigial. A espécie foi então separada em *B. arachnoides* arachnoides (presente nos estados do Rio de Janeiro, São Paulo e Paraná, ao longo da Serra do Mar) e *B. arachnoides* hypoxanthus (presente no sul da Bahia, Minas Gerais e Espírito Santo). Lemos de Sá *et al.* (1990, 1993) e Lemos de Sá & Glander (1993) concluíram que a diferenciação indicada por Vieira (1944) era ainda mais extrema, o que justificaria a separação de *B. arachnoides* em duas espécies: o muriqui do sul, *B. arachnoides* (É. Geoffroy, 1906) e o muriqui do norte, *B. hypoxanthus* (Kuhl, 1820) (Groves, 2001). Este trabalho se refere à espécie de muriqui do sul, *B. arachnoides.*

A área de vida do muriqui do sul é situada em altitudes de 600 a 1.800 m acima do nível do mar, em remanescentes florestais nos estados de São Paulo (limite norte é a Serra da Mantiqueira; Lemos de Sá & Glander 1993), Rio de Janeiro e Paraná (limite sul é a região do Rio Ribeira; Aguirre, 1971).

B. arachnoides é considerada a maior espécie de primata neotropical, pesando entre 9,4 e 12,1 Kg (Reis *et al.,* 2006). Indivíduos dessa espécie formam grupos que variam de 20 a 60 indivíduos, os quais incluem vários machos, fêmeas, filhotes e jovens que convivem pacificamente (Dias & Strier, 2003; Talebi *et al.,* 2005). As fêmeas dispersam do grupo natal por volta dos seis anos de idade, enquanto os machos permanecem no grupo (Strier *et al.,* 2002). A sua dieta consiste basicamente de frutos, folhas e flores e sementes (Talebi *et al.,* 2005), embora, de acordo com Martins (2005) existam diferenças entre as porções desses itens dependendo do tipo de floresta em que os muriquis se encontram (semi-decídua, *evergreen* ou ainda fragmentada). Esta plasticidade os torna bem adaptados às mudanças estruturais e floristicas das últimas décadas. É possível que essas estratégias alimentares forneçam vantagem ao muriqui

para tolerar não somente repetidas estações secas, como também efeitos antropogênicos sobre os remanescentes de floresta em que eles vivem (Martins, 2005).

Strier (1997) estudou o padrão de cruzamentos em duas populações de *B. hypoxanthus*, e seus resultados indicam que cada fêmea copula com muitos machos durante o período pré-ovulatório. Apesar de as fêmeas copularem com múltiplos parceiros, raramente foram observadas cópulas entre mães e seus filhos adultos. Devido à promiscuidade do sistema de cópula dos muriquis é impossível determinar a paternidade dos infantes sem estudos genéticos, já que estudos de comportamento revelaram uma grande tolerância dos machos adultos com todos os infantes. Esse tipo de comportamento também pode ser atribuído à espécie *B. arachnoides*.

O primeiro estudo envolvendo genética dos muriquis foi realizado por Pope (1998). Este trabalho avaliou a variabilidade genética em 32 *loci* de aloenzimas em duas populações de muriquis, uma na Fazendo Esmeralda, ao leste do estado de Minas Gerais, e outra na Fazenda Barreiro Rico, no leste do estado de São Paulo. Neste trabalho, Pope considera os indivíduos dessas populações como sendo da mesma espécie, e registra altos níveis de diferenciação genética entre eles (34,4% de *loci* polimórficos e 11% a média de heterozigosidade por lócus nas duas populações), os maiores já registrados para espécies de primatas neotropicais. Esse resultado atípico foi provavelmente devido ao fato de que os indivíduos das duas populações pertencem a espécies diferentes. Até agora não existem estudos genéticos envolvendo apenas a espécie *B. arachnoides*.

O histórico de desmatamento da Floresta Atlântica, desde 1500, tem reduzido a mata a pequenos fragmentos com pouca ou nenhuma conexão entre eles. Isso resultou em poucas, pequenas e esparsas populações de muriquis. A falta de conectividade entre essas populações tem eliminado a oportunidade de fluxo gênico e migração entre elas (Fagundes, 2005).

Muitas das características demográficas e ecológicas de *Brachyteles*, tais como pequenas e isoladas populações, longo intervalo entre nascimentos, taxa de maturação lenta, e gerações longas e sobrepostas fazem dele um gênero muito vulnerável aos efeitos da deriva genética e intercruzamento, com vários riscos de baixa variabilidade genética e baixa viabilidade (Strier, 2000; Strier *et al.,* 2002).

O objetivo deste trabalho foi avaliar o padrão filogeográfico, diversidade genética e história demográfica da espécie *B. arachnoides* em toda a sua distribuição utilizando para isso marcadores moleculares do DNA mitocondrial (mtDNA) e do DNA nuclear (microssatélites).

2. Artigo

Phylogeography of Southern Muriqui, *Brachyteles arachnoides* (Primates, Atelidae)

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Introduction

Compared with others biomes of Brazil, the Atlantic Forest is the most diverse for its size and degree of endemism, estimated at 32% (Costa *et al.,* 2000). The original area covered by the Atlantic Forest when the European colonization began in 1500 was of *ca* 1,300,000 km^2, spreading from Rio Grande do Norte state, at the easternmost tip of South America, to the southermost tip of Rio Grande do Sul, the southernmost Brazilian state (Collins 1990). The unplanned occupation of the forest caused a reduction to about 8% in its original extent (Ministério do Meio Ambiente 1999). This forest destruction has resulted in the elimination of many populations and potentially in the erosion of the genetic diversity of several species (Brown & Brown 1992). One of the biggest conservation problems of the Atlantic Forest is the habitat fragmentation, i.e., the process of reduction and isolation of a plant population that can affect all the natural populations existing within this habitat (Scariot *et al.,*2003).

The two species of the atelid genus *Brachyteles* are the largest New World monkeys and are distributed entirely on the Atlantic Forest: *Brachyteles hypoxanthus* or northern muriqui and *B. arachnoides* or southern muriqui. This paper focuses on the phylogeography and conservation genetics of the southern muriqui. The southern muriqui form groups of males and females, young and juveniles of various sizes, depending of the area's size (Strier, 1997). The mating system is promiscuous (Strier, 1997) and the females transfer out of the natal groups with about six years old, prior to becoming sexually active (Strier, 2005) and give birth to their first infants at about nine years old (Strier & Ziegler, 2000). Studies suggested (e.g. Milton, 1984) that the major part of the *B. arachnoides* diet consists of leaves, although fruits may constituted a significant proportion of their observed diet (e.g. Fonseca, 1985) and may surpass the amount of leaves in some periods of the year, when fruits are more abundant.

The original range of the southern muriqui goes through two forest types in the Atlantic forest: the broadleaf evergreen forests on the eastern slopes of the Serra do Mar in the states of Rio de Janeiro and São Paulo, and the mesophytic, semideciduous forests on the central plateau, in the interior of the states of São Paulo and Paraná (Martins, 2005). The ongoing process of habitat fragmentation drastically reduced the populations of southern muriquis that now survive only in the largest preserved areas

and a few forest fragments (Martins, 2005), being listed as endangered in the red list of IUCN (http://www.iucnredlist.org/apps/redlist/details/2993/0). According to Talebi & Soares (2005) the greatest threats to the species, beyond forest fragmentation are hunting and mining in areas of occurrence of populations. In addition some demographic and ecological features of *Brachyteles* species, such as small and isolated populations, long interval between births, slow rate of maturation and long and overlapping generations make then very vulnerable to the effects of genetic drift and interbreeding, with serious risks of reduction of genetic variability and viability (Strier, 2000; Strier *et al.,* 2002).

Unfortunately, no molecular population genetics study has been done so far in the southern muriqui, while several genetic studies were published for the northern muriqui (e.g. Fagundes *et al.,* 2008), including a recent large phylogeographic assessment using mtDNA sequencing (Chaves *et al.* 2011). Besides the long term evolutionary history depicted by mtDNA data, the impact that recent anthropogenic alterations to the Atlantic forest and *B. arachnoides* populations should be better appraisal using additionally a more variable marker such as autosomal microsatellites. So the purposes of the present study were to describe the patterns of mtDNA and microsatellite genetic diversity and their bearing on the geographic structure and demographic history of *B. arachnoides*.

Materials and Methods

Sampling and laboratory techniques

The samples used in this work are mostly feces of wild animals and some additional blood samples from captive animal from known origin. In total we analyzed sixty (60) samples from most of the species´ distribution (states of Paraná, São Paulo and Rio de Janeiro (Figure 1, Table 2). The samples were extracted with appropriate protocols according to the referenced material (Paternity Testing Procedures for Use with the Life Tissues, prepared by FBI; QIAmp DNA Stool Mini Kit from Qiagen). The extractions of DNA from feces were performed in a dedicated isolated room with specifications similar to an ancient DNA room.

Approximately 600 bp from the mtDNA control region were amplified with the primers HowRa1 (Ascunce *et al.*, 2003) or RC_Brachy_F (5'-TTCTAAATTAAACTACTCCCT-3',designed here) and RC_Bugio (Jerusalinsky, 2001). With the DNA extracted from feces, a nested PCR was performed with the internal primers Int_RC_Brachy_F (5'-AGCCATCTAACACGTAAATCC-3') and Int_RC_Brachy_R (5'- CCAGCGTAATGTGCTATGT 3'), designed for us. The PCR reaction contained around 10ng of DNA, 0,25ul of each primer, 1X buffer, 1,5mM MgCl2, 0,1mM dNTPs, 0,4 units of platinum Taq polymerase and 0,1mg/ml BSA. The first amplification started with an initial denaturation at 94ºC (2 min), followed by 7 cycles at 94ºC (30 s), 55ºC -1ºC/cycle (60 s) and 72ºC (60 s) and 40 cycles at 94ºC (30 s), 50ºC (60 s), 72ºC (60 s) and finished with the final extension at 72ºC (5 min). Amplifications with internal primers used only 30 cycles with annealing temperature of 50ºC. Amplified fragments were sequenced using the DYEnamic ET Dye Terminator Cycle Sequencing Kit (Amersham Biosciences). Chromatograms were checked with the Geneious software 5.4 (Biomatters LTDA) and the sequences were manually edited using Bioedit 7.0.9 (Hall 1999).

Fourteen microsatellites (113, 1110, 1115, 1118, 157, APM1, AB06, D8S165, D8S260, SB30, D17S804, LEON15, LEON21 and LOCUS5), developed originally for other species of primates, were genotyped for fifty five (55) individuals. These primers were selected according to the results of variability and efficacy tests, results that proved that these primers were useful for this type of study (Strier *et al.* in prep.). The PCR reactions were performed according to the protocol of QIAGEN Multiplex PCR Kit, with primers concentrations range of 0.04uM to 0.22ul (see supporting material, table S1). Two amplification conditions were used to amplify the entire primers set:

- The first amplification procedure started at 95ºC for 15 min followed by 10 cycles at 94ºC (30 s), 60ºC -1ºC/cycle (90 s), 72ºC (90 s) and 30 cycles at 94ºC (30 s), 50ºC (90 s), 72ºC (90 s) and finished with a final extension at 60ºC (30 min).

- The second amplification condition started at 95ºC for 15 min followed by 35 cycles at 94ºC (30 s), 55ºC (90 s), 72ºC (90 s) and finished with a final extension at 60ºC (30 min).

Microsatellites genotyping were performed in MegaBACE 1000 automated sequencer (Amersham Biosciences) according to the manufacturer's protocols.

Analyses

Mitocondrial DNA

Sequences were aligned with the ClustalW algorithm in Bioedit and adjusted manually. Since for most samples we do not have exact geographic location, latitude and longitude were inferred using the geographical center of the town where the samples were collected. Relationship between the haplotypes were estimated by the software Network 4.6 (http://www.fluxus-engineering.com/sharenet.htm) with the median-joining method (Bandelt *et al.,* 1999). For this analysis we used only the individuals from which the first half of the region, the most polymorphic, was obtained (see results). Basic diversity statistics such as Tajima's (Tajima 1983) and Fu (Fu 1997) neutrality tests, F-statistics (F_{ST}; Hudson *et al.,* 1992) and haplotype and nucleotide diversity were estimated using DnaSP 5 (Librado & Rozas, 2009) and Arlequin 3.5.1.3 (Excoffier & Lischer, 2010).

Population size dynamics through time was inferred using GMRF Bayesian skyride plots (BSP) (Minin *et al.,* 2008) as implemented in BEAST 1.6.1 (Drummond & Rambaut , 2007). Beast was run for 20 million iterations sampling each 2,000 chains and the first 10% iterations were discarded as burn-in. The runs were visually inspected using TRACER 1.5 (http://beast.bio.ed.ac.uk/Tracer). Bayesian skyride plots used a time-aware smoothing and a substitution rate of 2.815×10^{-7} (confidence interval 95% 1.459×10^{-7} - 4.399×10^{-7}) as estimated for the Platyrrhini mtDNA control region by Machado (2011). A generation time of 15 years was used (Strier, 2005).

Isolation by distance was tested using the Mantel test (Mantel, 1967) implemented in the software Alleles in Space 1.0 (AIS; Miller, 2005).

Microsatellites

Basic genetic diversity statistics such as the number of polymorphic loci, number of alleles for locus, expected (He) and observed (Ho) heterozigosity and Hardy-Weinberg equilibrium were estimated with Arlequin 3.5.1.3. Linkage disequilibrium between the loci was tested with the program GENEPOP 4.1 (Rousset, 2008).

The graphical method implemented in the software Bottleneck 1.2.02 (Cornuet & Luikart, 1997) was used to test the existence of a past genetic bottleneck. The parameters used were 1000 iterations using stepwise mutation model (SMM; Ohta & Kimura, 1993) along with a percentage (5%) of two-phase model (TPM; Di Rienzo *et al.,* 1994).

The program LDNe (Waples & Do, 2008) was used to estimate a 95% confidence interval of the effective population size (N_e) through the LD (linkage disequilibrium) method, which infers the N_e of a parental generation of the individuals sampled. The program ONeSAMP (Tallmon *et al.,* 2008) is a web-based program that uses an approximate Bayesian computation to estimate the effective population size (Tallmon *et al.,* 2008). To estimate the Ne with this program 50 individuals and 10 loci (all loci with more than 20% of missing data and the monomorphics were removed to run the simulation) were used. The limits of the effective population size used in a first run were between 2 and 1,000 and in the final run between 2 and 200.

The program STRUCTURE 2.3.3 (Pritchard *et al.,* 2000, Falush *et al.,* 2003, Falush *et al.,* 2007, Hubisz *et al.,* 2009) was used to infer the population structure of the species and to connect individuals with their population of origin. Ten iterations and 1 million of MCMC (the first 10% were discarded as burn-in) were done to test the K values from 1 to 10. In order to sort our results, we derived symmetric similarity coefficients among replicate runs within each value of *K*, following Wang *et al.,* (2007). This was undertaken using the Greedy algorithm of Clumpp (Jakobsson & Rosenberg, 2007). Summary outputs for each value of *K* were then displayed graphically using the software Distruct (Rosenberg, 2004).

Results

Mitochondrial DNA

An alignment of 612 bp of mtDNA control region was obtained of 60 individuals of *B. arachnoides*. Thirty four variable sites were found, being 24 transitions, 2 transversions, 8 indels and an insertion of 43 bp in one individual, resulting in 39 haplotypes. The haplotype diversity (H) was 0.976 and the nucleotide diversity (π) was 0.976%. This reasonable diversity could be observed in the network (Figure 2) that

shown haplotypes separated by several substitutions. Also noteworthy was the lack of clear geographical structuring in the network. This could be showed by our best sample site (PECB) that present high number of haployptes that are distributed all over the network. On the other hand, only the most frequent haplotype (H2) is shared between individuals from three different locations, all from the southern part of the distribution. Some structuring could be found with the clustering of the individuals of the PNSO (RJ), although together with some haplotyes from distant individuals. The Mantel test were not significant (r=0.01, p= 0.55), suggesting no evidence for a clear relationship between genetic and geographic distances.

The neutrality tests for the whole species suggest a population demographic expansion, since the Fu's Fs test was negative (-24.218) and significant (p=0.000), and Tajima's D was also negative (-1.142), although not significant (p= 0.11). The Bayesian Skyride Plot (figure 3) is also consistent with a population expansion of ~10 times, the female effective size increasing from ~1,500 to ~15,000 around 15 thousand years ago (kya). The graphics also suggest some slow population reduction from the past toward 15 kya. However, confidence interval is mostly broad; the credibility interval of the current Nef was 5,000 to 100,000, so the above values should be regarded with prudence.

Microsatellites

A total of 55 individuals were genotyped for 14 microsatellite loci. Of the 14 loci analyzed, 13 were polymorphic, with the number of alleles per locus ranging from 3 (D8S260 and 1115) to 12 (AB06) with an average of 6.86. The expected heterozygosity ranged from 0.037 (D8S260) to 0.6182 (SB30) and the observed heterozygosity ranged from 0.074 (D8S260) to 0.778 (SB30). The results for each locus are showed in table 1.

The software Structure suggested that the best K number of clusters for the individuals sampled was 4 (figure 4), according to deltaK and L(K). However, these groups are not clearly defined, with most individuals presenting part of their genetic ancestry in different clusters. Besides, similarly to the mtDNA network, there is no clear geographic structure in the cluster. Because of these results, most of our further analyses would consider the whole species as a single population.

There is no evidence of a recent genetic bottleneck using the graphical method in Bottleneck (See Suporting Material, Figure S1). The program LDNe estimated the effective size of *B. arachnoides* between 35 and 112 individuals according to the confidence interval (95%) of the jackknife method, with the mean of 56 individuals, using the smaller allele frequency of 0.02. The program ONeSAMP estimated a mean Ne of 75 individuals, ranging from 50 to 151 (95% CI).

Discussion

Both mtDNA and microsatellite markers are concordant suggesting that the southern muriqui presents a relatively high and geographically unstructured genetic diversity. Besides, there is no evidence for any recent (anthropogenic) significant reduction in the genetic diversity, and the mtDNA data suggested a significant population size increase since around 15 kya.

The absence of significant geographic structure is not unexpected, in special regarding mtDNA, since females are known to disperse from their natal group (Milton, 1984; Strier, 2005) and given its lower mutation rate usually reflects more ancient patterns (see below). Besides, the absence of geographic structure may be a consequence of the relatively recent (in evolutionary times) population expansion estimated using BSP (see below). On the other hand, given that microsatellite loci usually respond more rapidly to more recent fragmentation, one would expect they would show at least some evidence of geographic structure caused by the recent and dramatic fragmentation of the Atlantic Forest. However, the southern muriqui usually occupy areas that are still relatively large and continuous in the least deforestated region (National Muriqui Action Plan, 2011- PAN 2011) and these seem to have preserved the historical non-structured nature of their genetic diversity. It seems that even the genetic diversity of the northern muriqui, which occurs in much more fragmented areas, was not yet much affected by these recent events (Chaves *et al.,* 2011).

The Bayesian Skyride Plot (figure 3) of the southern muriqui mtDNA is broadly consistent with scenario of a strong influence of the Late Quaternary climate fluctuations to the long term population size dynamics of Atlantic forest species (see Carnaval &

Moritz, 2008). This hypothesis suggests the existence of refugia, areas forest stability during glacial times, in special the Last Glacial Maximum (LGM, ~25-19 kya). The BSP show a slow population reduction from the past (during glacial age) to the LGM, when population reached its minimum; after that, population expanded ten times to current size. It should be noted that despite the size reduction during the last glacial age, southern muriqui seems to have maintained a population of reasonable size, since we could not detect a clear genetic bottleneck in our data. Actually, most of the area of occurrence of the southern muriqui is within the putative São Paulo refugium (Carnaval & Moritz 2008). Moreover, several studies showed the occurrence of Myrtaceae and Melastomataceae (Behling & Negrelle 2001; Pessenda *et al.,* 2009) in the area since at least ~30 kya, both of which can serve as food for *B. arachnoides* (Martins, 2008), which suggest the existence of subtropical forests cold-adapted in periods before and during glaciations.

Noteworthy, the Bayesian skyline plot for the mtDNA data in the northern muriqui (Chaves *et al.,* 2011, their figure 5) shown a long term population stability, not showing any signal of reduction during the LGM as found here for the southern muriqui. The whole area of occurrence of the northern muriqui is in part of a predicted forest refugium (Bahia) that seems to be much larger and stable than the São Paulo refugium (Carnaval & Moritz 2008). Thefore, the northern muriqui may have maintained a larger population even during the LGM.

The mode of the current female effective population size of ~15,000 estimated using the BSP approach was very high compared with the microsatellites, but is similar to the 14,000 estimated to the northern muriqui (Chaves *et al.,* 2011). As noted by Chaves *et al.* (2011), although much higher than present day census size, the estimated pre-Columbian population size for both species was at least 400,000 (Aguirre 1971). Therefore, given the mtDNA substitution rate and the absence of any recent genetic bottleneck, estimates more likely reflects the average Nef of the last few millennia than the population reduction in the last century. On the other hand, the effective sizes of *B. arachnoides* estimated from the bi-parental microsatellites by the two methods were very similar, between 35 and 151 individuals. This is small, considering that the current census size (Nc) of the whole population is likely between 1,000-2,000 (PAN 2011). The

difference between the mtDNA and the microsatellite effective sizes could be partially explained since microsatellite frequency data is much more sensible to recent population changes, although we could not detect a significant signal of population reduction with this data. The difference between the microsatellite Ne and the Nc could be explained by several factors. For example, the historical effective size is usually lower or much lower than current census size because Ne is close to the harmonic mean of sizes over generations and it is especially sensitive to the lowest values (Hartl & Clark, 1997). Besides, our individuals came mainly from the PNCB area, which population size is estimated between 500 and 800 individuals (PAN 2011), and so our estimates may reflect a more local size estimate.

An important aspect of this relatively high genetic diversity and absence of genetic bottleneck is that they also suggest that despite the recent devastation of the Atlantic forest, into which less than 8% of the original vegetation remains (Ministério do Meio Ambiente, 1999), the southern muriqui seems to have maintained much of its historical genetic diversity. A similar pattern was found for the more threatened northern muriqui (Fagundes *et al.,* 2008, Chaves *et al.,* 2011). As noted above, for the southern muriqui its occurrence in forested areas that are relatively large and still maintain some connections may explain the absence of any clear anthropogenic genetic bottleneck in the species. Of course the persistence of the current deforestation rate may severely change this situation in the near future.

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Legends for figures

Figure 1. Map showing the sampling sites and the historical distribution area (dotted line) of *B. arachnoides* . 1- Parque Nacional da Serra dos Órgãos (Soberbo), 2- Parque Nacional da Serra dos Órgãos (TP), 3- Parque Nacional da Serra dos Órgãos (Cachoeira Véu da Noiva), 4- Parque Nacional de Itatiaia, 5- Serra da Mantiqueira, 6- Vale do Paraíba, 7- Bertioga, 8- Mongaguá, 9- Juquitiba, 10- Parque Estadual Carlos Botelho, 11- Parque estadual do Petar, 12- Morretes..

Figure 2: Median-joining network of the mtDNA *B. arachnoides* haplotypes. The color of the circle represents a sample points. The size of the circle is related to the frequency of the haplotype in the different sampling sites (color according the legend). The small red squares represent median vectors. Dashes in the lines connecting the haplotypes represent inferred substitutions.

Figure 3: Bayesian Skyride plot of the mtDNA data showing the effective population size fluctuation throughout time (in years). The black line represents the posterior median values and the blue area the 95% credible interval.

Figure 4: Bayesian admixture proportions of individuals of *B. arachnoides* using 14 loci as performed by Structure. Each individual is represent by one thin line which K segments colored proportionally according to their ancestry in the genetic clusters (K=4).

Figures and tables

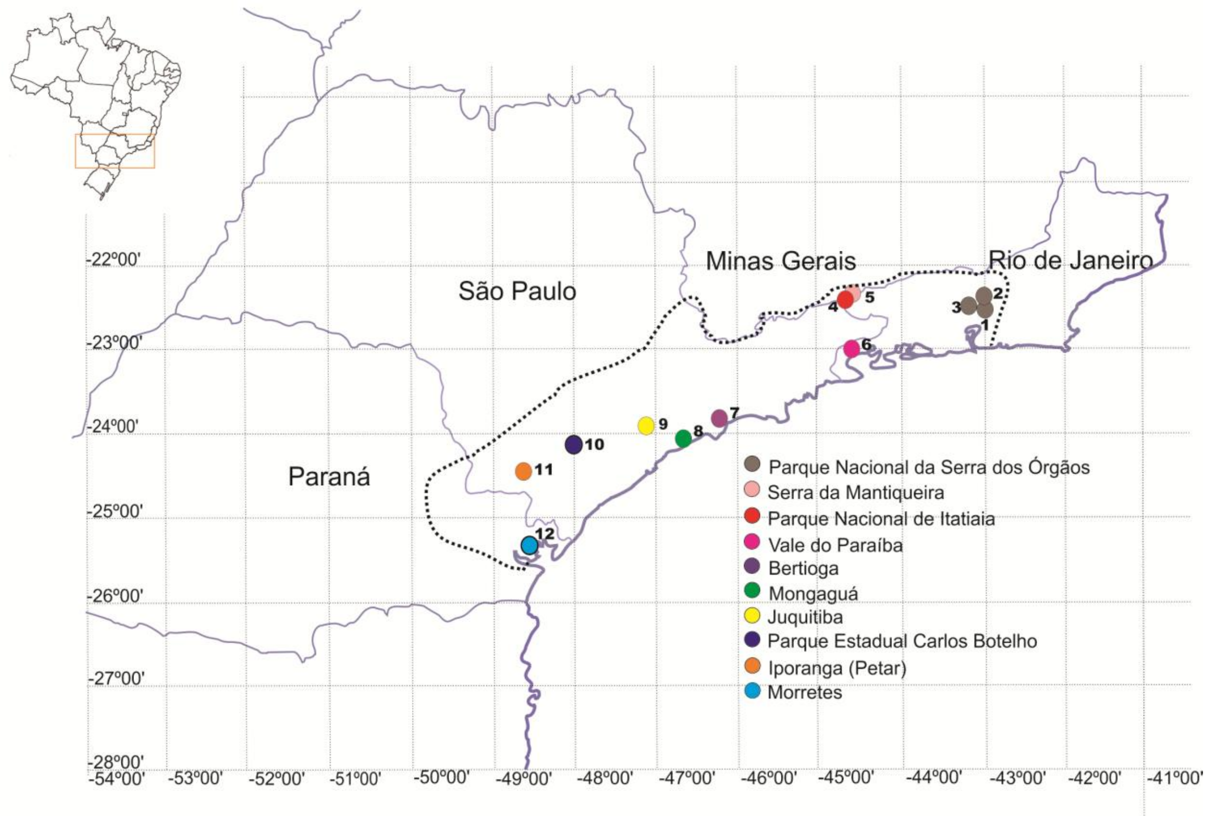

Figure 1

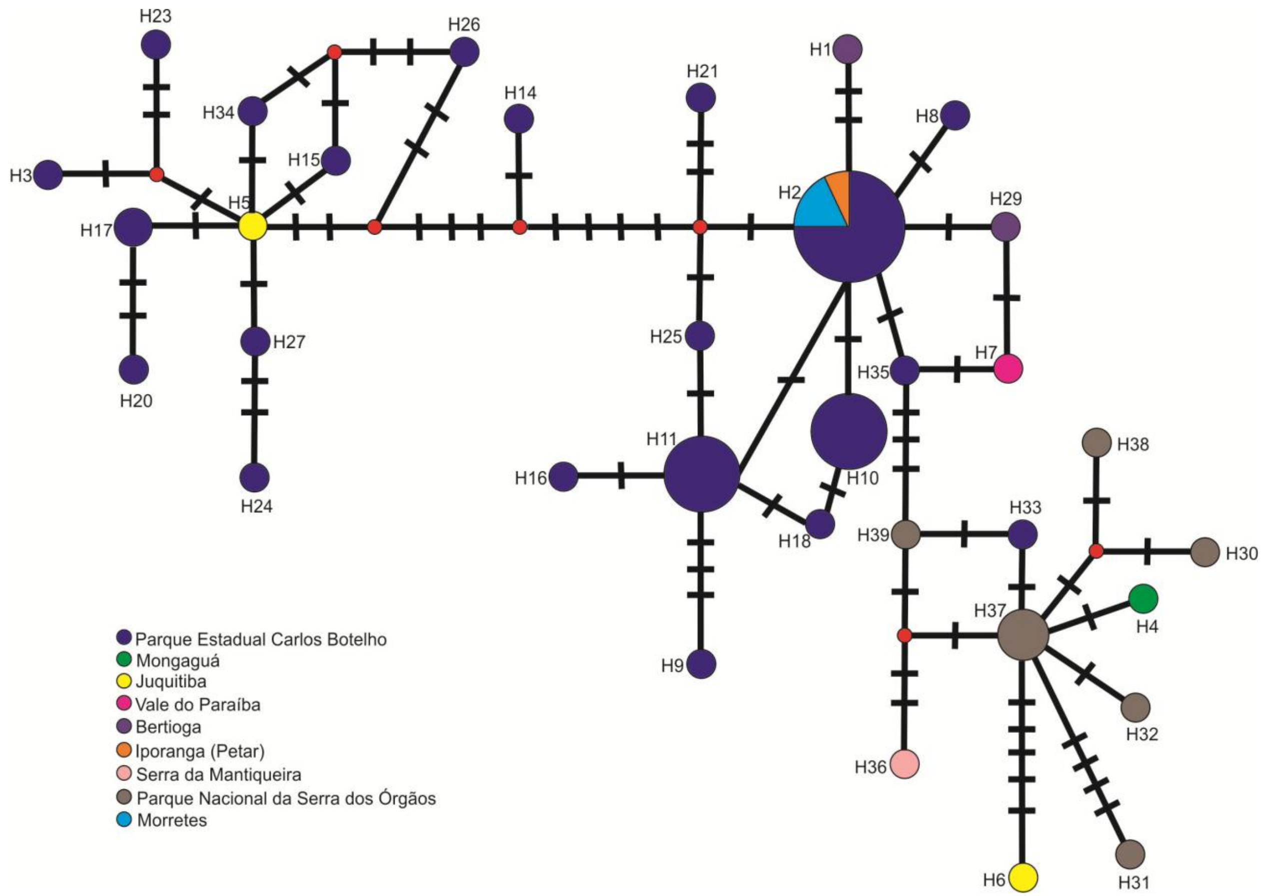

Figure 2

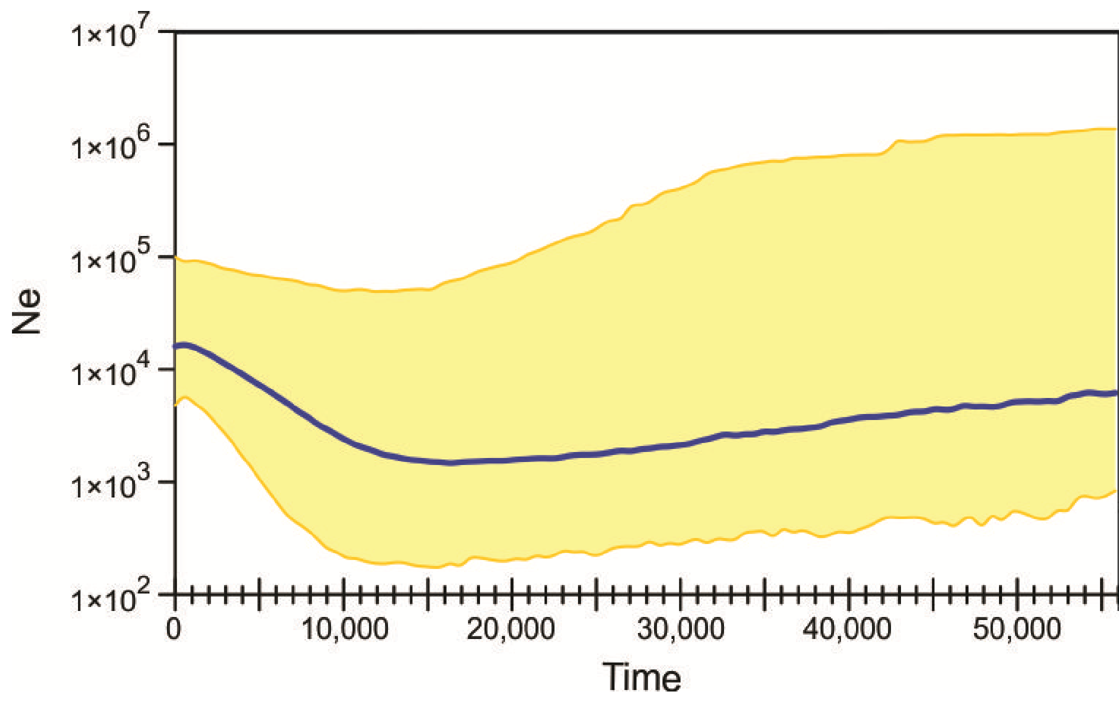

Figure 3

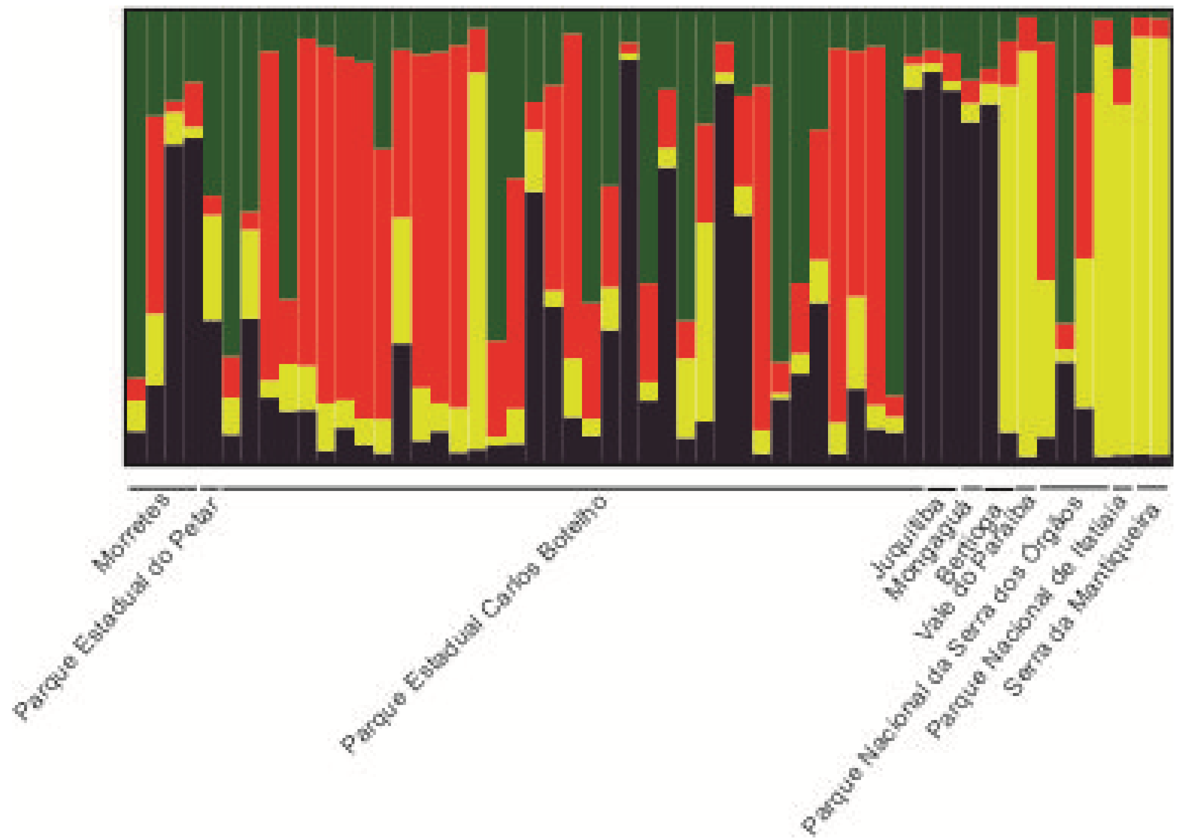

Figure 4

Table 1: Characterization of microsatellites markers in *B. arachnoides*, including marker source, primers sequence, repeat type, molecular size range in base pairs and diversity parameters: number of alleles, expected (He) and observed (Ho) heterozigosity.

Locus	Primers (5'-3')	Repeat motif	Size range in *B. arachnoides*	Num Alleles	He	Ho
1118 [1]	TTTCTCCCTCTCAGATTACCAG CCTTGAGGTTTTTGGGTTCC	di	161 - 181	6	0.58886	0.56604
D8S260 [2]	GATAAGGTTGCTTATTTTTGAGTCTG TTGTCTGCTGAAGGCTGTTCTATG	di	203 - 209	3	0.07403	0.03774
1115 [1]	GCTCATATTCATACATCCCTTGG TTTGCTTCGTCATTCATTGC	di	196 - 206	3	0.35944	0.14286
157 [1]	TGGCAAGTCTGGTTTCAAGC TTCCAGACTGAGCTAGGATGC	di	243 - 257	7	0.66207	0.34483
D8S165 [3]	ACAAGAGCACATTTAGTCAG AGCTTCATTTTTCCCTCTAG	di	127 - 147	6	0.47772	0.36842
D17S804 [2]	GCCTGTGCTGCTGATAACC CACTGTGATGAGATGTCATTCC	di	153 - 175	8	0.75625	0.47368
1110 [1]	GGTGAATGAGAGAATCAAAG TATGTTCCACAGTAGAAAGC	di	207 - 233	9	0.70139	0.43478
SB30 [4]	TAAAGTTAAGATTGGATTTCAC GCAGAAAAACCTAACAATACA	di	91 - 109	8	0.77832	0.61818
113 [1]	GCAAAACTCCCCTGTGACTG CCCACTCTCCTCCACAAAGG	di	200	1		
LEON15 [5]	CTGATCCTTGAAGCAGCATTG GGTTAAAGGGGTTCGTTCTGTG	di	257 - 279	6	0.73369	0.30189
LOCUS5 (LrP2BH6) [6]	TCTGTTTGAATCCCCAGTCC GCAGTCCCTCAAGGTTTTCT	di	100 - 114	8	0.66667	0.29167
APM1 [7]	CACGTGTGTCCAGCTTGTCT ATTCTGCTGCCCTTGAGTTC	di	200 - 216	7	0.73028	0.29091
LEON21 [5]	CAGTTGAGGGAACAGGAATTA CACTGCACTGACAGAGCAAG	tetra	359 - 383	5	0.50286	0.39024
AB06 [8]	GTGATTATTGTGTGGTACTTG ATGTATTTTTCTGGTTTTACC	di	258 - 318	12	0.73015	0.31373

The numbers above the name's locus mean: 1- Di Fiore & Fleischer 2004; 2- Weissenbach *et al.,* 1992; 3- Weber & May 1989; 4- Bohle & Zischler 2002; 5- Perez-Sweeney *et al.,* 2005; 6- Grativol *et al.,* 2001; 7- Cortes-Ortiz *et al.,* 2010; 8- Gonçalves *et al.,* 2004.

Table 2: Number of individuals for sampling point.

Region	Sampling point	Number of individuals
Rio de Janeiro	Parque Nacional da Serra dos Órgãos	16
	Parque Nacional de Itatiaia	2
	Serra da Mantiqueira	2
São Paulo	Vale do Paraíba	1
	Bertioga	2
	Mongaguá	1
	Juquitiba	2
	Parque Estadual Carlos Botelho	37
	Parque Estadual do Petar	1
Paraná	Morretes	4

Supporting material

TableS1. Primer sets for each multiplex and amplification conditions. * see table S2.

Primer set	Loci 1	Loci 2	Loci 3	Primer concentration (uM)	cycling conditions*
Multiplex 1	1118	D8S260		0,1- 0,1	PCR1
Multiplex 2	1115	157		0,14- 0,08	PCR1
Multiplex 3	D8S165	D17S804	1110	0,04- 0,11- 0,22	PCR1
Multiplex 4	SB30	113	Leon15	0,13 -0,04 -0,06	PCR1
Multiplex 5	Locus 5	APM1	AB06	0,14 -0,08 -0,08	PCR2
Multiplex 6	D13S160	Leon21		0,1 -0,1	PCR2

TableS2. Details of the PCR cycles.

	Cycles	Temparature	Time
PCR1	1	95ºC	15'
	35	94ºC	30''
		55ºC	90''
		72º	90''
	1	60ºC	30'
PCR2	1	95ºC	15'
	10	94ºC	30''
		60ºC (-1º/cycle)	90''
		72ºC	90''
	30	94ºC	30''
		50ºC	90''
		72ºC	90''
	1	60ºC	30'

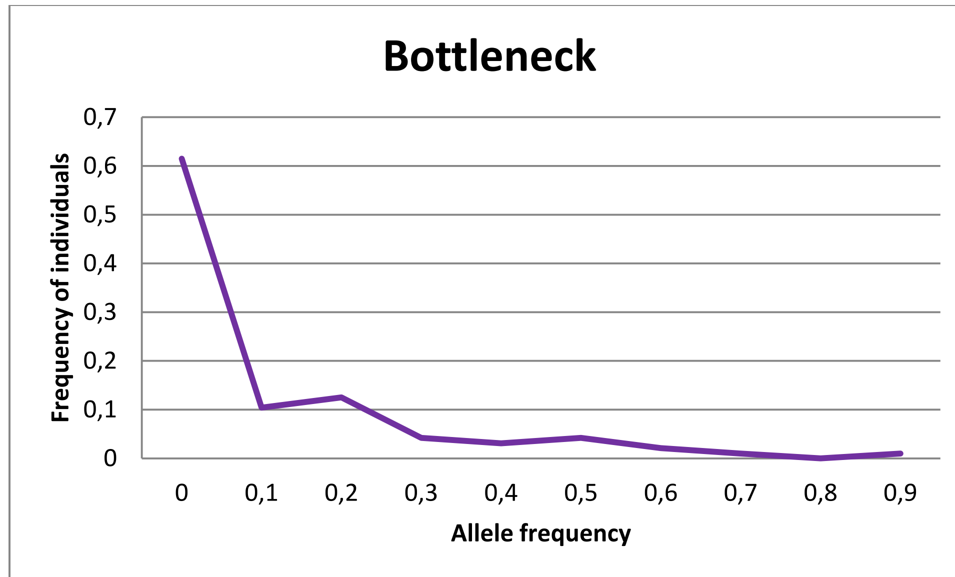

Figure S1: Distribution of allelic frequency classes. An L-shaped curve suggests population stability since a large amount of individuals still retains alleles with low frequency, which are the first to be lost when there is a bottleneck.

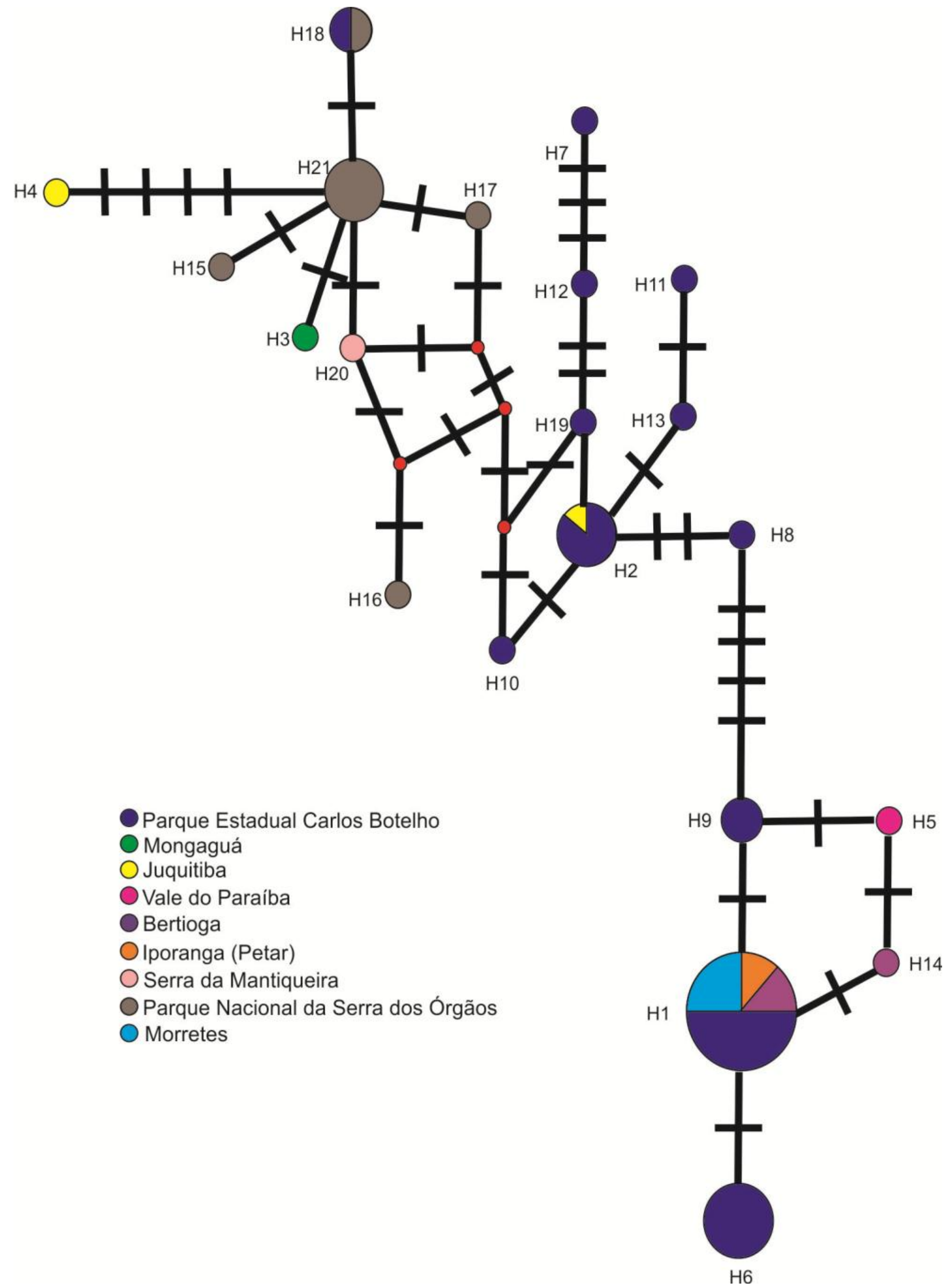

Figure S2: Median-joining network as fig. 2 but based on the alignment without gaps.

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