University of Salford

School of Environment & Life Sciences

Non-invasive monitoring for population assessments of a critically endangered Neotropical primate

This thesis is submitted to University of Salford, School of Environment & Life Sciences, in fulfilment of the requirements for the degree of Doctor of Philosophy (PhD).

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“We have the choice to use the gift of our life to make the world a better place – or not to bother”

Jane Goodall

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Abstract

The northern muriqui (*Brachyteles hypoxanthus*), is a Critically Endangered primate endemic to the Atlantic Forest of Brazil. Remnant populations of muriquis suffer from a wide range of anthropogenic pressures and face a high risk of extinction due to demographic and environmental stochasticity. Despite the conservation status of the species and its urgent need for management and conservation based on scientific evidence, some muriqui populations are poorly studied and/or remain neglected due to the difficulties of applying traditional survey methods. Therefore, in this thesis, I assessed and provided population and genetic data of a muriqui population based in a previously unstudied site using remote sensing and molecular methods. I evaluated the use of canopy camera traps as an effective tool to detect and for a population assessment of non-habituated groups of muriqui and other arboreal primates (*Callithrix flaviceps* and *Sapajus nigritus*) in remote areas, demonstrating the applicability of the method for Neotropical primate surveys and conservation. Non-invasive genetic sampling allowed for a complementary population assessment in northern muriqui and provided information regarding the genetic diversity of the species in comparison to neighbouring populations. Overall, my studies demonstrated the potential and the effectiveness of an integrative approach based on non-invasive methods to advance our knowledge regarding a neglected and poorly studied population of muriquis, including recently discovered groups. The information provided here strengthens the potential of implementing non-invasive techniques for the assessment and monitoring of Neotropical primates in remote and difficult to reach areas, with the ultimate goal of informing management decisions and promoting species conservation.

Keywords: arboreal camera trapping, *Brachyteles hypoxanthus*, conservation genetics, primates

Chapter I

INTRODUCTION AND BACKGROUND

Chapter I

Introduction and Background

Nonhuman primates (primates hereafter) are been facing extinction threats worldwide. A recent evaluation shows that populations from 75% of global primate species are now declining, and more than half of the species are facing near-term extinction (Estrada et al. 2017). Despite the rarity of some taxa and the intrinsic aspects of some species biology, habitat loss due agriculture as well as hunting are the major global threat to primates (Estrada et al. 2017). Forest loss associated to habitat fragmentation and degradation potently affects forest-dwelling species, and those arboreal species are more impacted by human forest disturbances than terrestrial ones due the loss of arboreal connectivity (Whitworth et al. 2019). Frequently, forest losses are accompanied by hunting and by a range of secondary threats (i.e. livestock farming, logging, mining, fossil fuel extraction, diseases, illegal trade, invasive alien species, and climate changes) and challenges the survival of most species, especially those endangered and critically endangered (Wich & Marshall 2016; Estrada et al. 2017, 2018; Graham et al. 2016, Carvalho et al. 2019). Since these pressures do not occur separately and have been increasing at a fast pace, there is a growing concern by conservationists to find practical solutions to monitor wild primate populations in order to protect primates effectively, prevent population losses and the extinction of species (Nichols & Williams 2006; Campbell et al., 2016).

Primate conservation and management actions in Neotropical forests are challenging mainly due to limited information to base management on. This is a result

to the difficulties in surveying arboreal primates from the ground, the limitations to access remote and densely forest areas (and often financial constraints). Most survey studies on arboreal primates rely on ground-based techniques, such as line transects (Buckland et al., 2010; Ferrari et al. 2010, Peres 1999) and/or counting for primate calls (Aldrich et al. 2008, Gestich et al. 2017). These techniques often miss rare and elusive species and are likely to fail in remote habitats and difficult terrains (Kays & Allison, 2001; Bowler et al., 2017). In addition, direct observations of habituated groups, which are financially and labour intensive and may impose ethical constrains due the risks of diseases transmission, vulnerability to poaching (Williamson & Feistner 2011, Fedigan 2010; Wallis & Lee 1999) and environmental interventions (Strier et al. 2010). In the face of these challenges, distribution and population data (i.e. density, composition, demography) are missing for a wide range of arboreal primate species in the Neotropical region.

1.1 | Brazilian primate diversity: Northern muriqui (*Brachyteles hypoxanthus*)

Brazil is one of the four high-priority countries for primate conservation in the world (Estrada et a. 2018), harbouring 114 known primate species (IUCN 2019). One third of Brazilian primate species are now threatened with extinction, and over half of them occur in the Atlantic forest - a biome that has been reduced to less than 90% of its original area and holds one of the highest diversity of plants and vertebrates in the world (Meyers et al., 2000; Ribeiro et al., 2009). The Atlantic rainforest faces a crisis of historic proportions due the loss and fragmentation of the forest (Dean 1996), along an increasing pressure from a growing human population and their anthropogenic

activities (Galindo-Leal & Gusmão 2003). Most of the primate species endemic to Atlantic Forest are now restricted to small and isolated fragments, and exposed to demographic and environmental stochasticity, which may result in species loss and population decline. An example, are the muriquis (*Brachyteles* sp.) that once were widely distributed throughout the centre-south portion of Atlantic Forest (Aguirre 1971) and are now living in a dozen isolated and fragmented forests.

Also known as woolly spider monkeys, the muriquis are the largest primate in the Neotropical region (~1.0 m in body length, up to 15 kg; Lemos de Sá 1993; Fleagle 1999). The genus comprises two distinct species endemic to the Brazilian Atlantic Forest: the northern muriqui (*Brachyteles hypoxanthus*) and the southern muriqui (*B. arachnoides*). Both species are classified as EDGE species (*Evolutionarily Distinct and Globally Endangered species*) and are Critically Endangered due to mainly extensive habitat loss and hunting pressure, resulting in a population decline exceeding 80% over the past 70 years (Ferraz et al., 2019, Talebi et al. 2019).

Figure 1 shows the historic distribution and the current populations of both muriqui species. This present study focus on the northern muriqui (*B. hypoxanthus*), which has now less than 900 individuals surviving in a dozen isolated populations (ranging from 2 to 335 individuals), in small to medium-size fragments (44 to 50,000 ha) that differ in habitat characteristics and anthropogenic pressures (Da Silva Júnior et al., 2010; Jerusalinsky et al., 2011, Strier et al. 2017). Although 81% of the known population of northern muriqui lies in Conservation Units, approximately 67% of these units have less than 15,000 ha in area (Jerusalinsky et al., 2011). In addition, only four areas have the potential to maintain viable population of northern muriqui in long-term, and thus are considered as priority areas for the species conservation *in situ* (Melo et al., 2018).

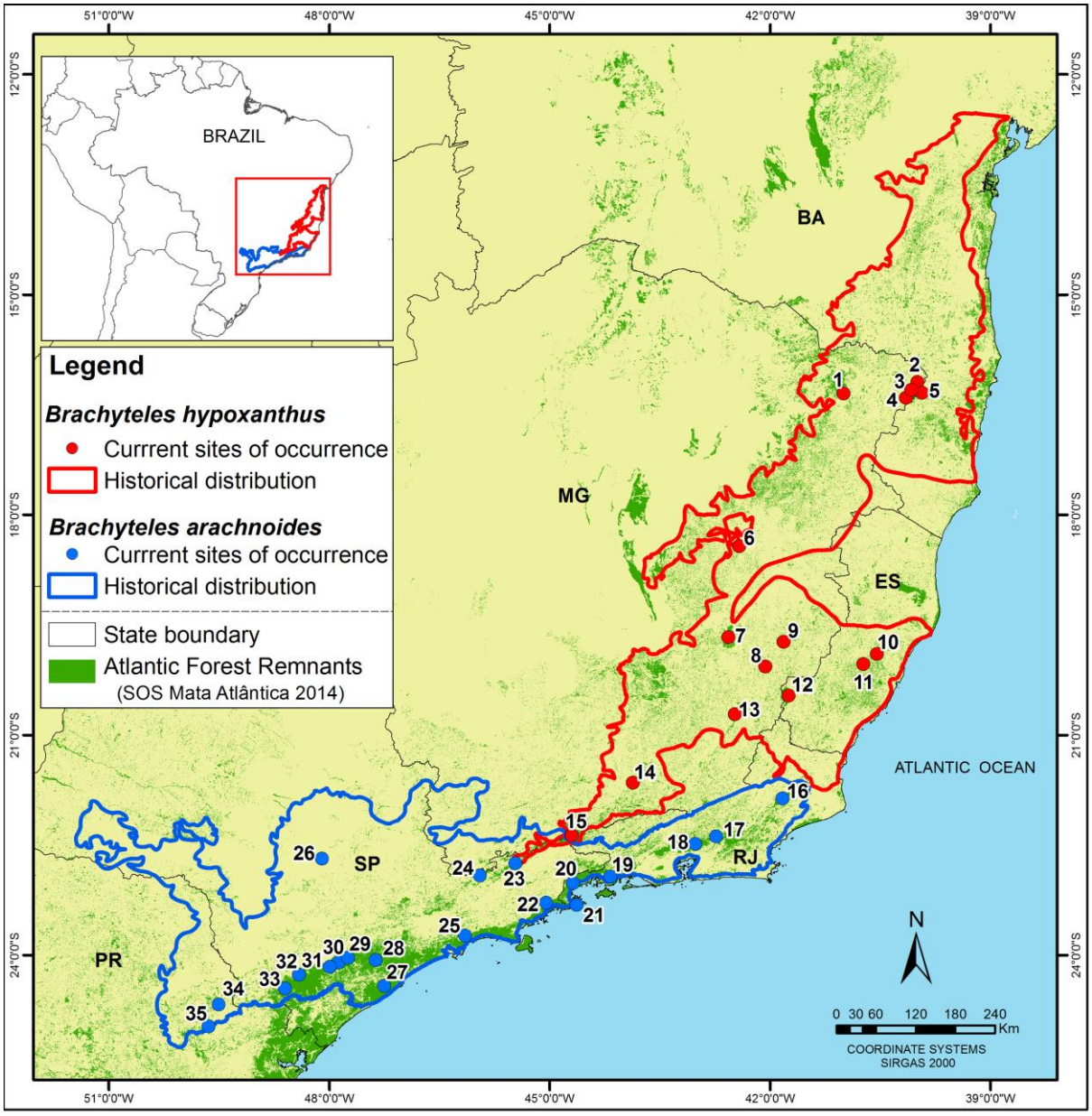


Figure 1. Historical distribution and known muriqui populations. Map from Strier et al. (2017)

With most of the remaining populations suffering from isolation and small sizes, they are probably not viable in long term or only viable with some sort of population management, such as the reintroduction or translocation of animals (Mendes et al., 2005). This scenario combined with the species' biology contributes to making the

muriquis more vulnerable to local extinction. Muriquis live in multimale-multifemale promiscuous groups, characterized by the philopatry of males while females disperse from their natal groups before the onset of puberty (Strier et al., 2006). Unlike other patrifocal primate species, such as chimpanzees and spider monkeys, the muriquis live in an egalitarian society without dominance and aggressiveness between members of the social group (Strier 1990, 1992). The northern muriqui has a slow life history, in general, the females begin to reproduce around nine years of age, and the intervals between births are approximately three years (Strier, 1999). These features associated with the existence of small isolated population, make muriqui groups susceptible to fixation of deleterious alleles by genetic drift, and promote inbreeding since this reduces and/or prevents the dispersion of females and, consequently, increases the degree of relatedness between individuals (Hedrick, 2000; Pope, 1998). In the short term, this process can reduce fitness components such as reproductive vigour and survival of individuals. In the long term, this reduces the adaptive potential of the populations in response to environmental changes, or resistance to new pathogens or diseases (Lacy, 1997; Frankham, 1998; Frankham et al., 2008).

Due to the increasing threats to northern muriquis and the critical status of the remaining populations, the requirement for management and conservation is more urgent than ever. In response to this situation, a National Action Plan for the Conservation of Muriquis (incorporated now in the National Action Plan for Atlantic Forest Primates and Maned Three-toed Sloth, PAN-PPMA) was enacted to reduce the significant risk of extinction of both muriqui species. The National Action Plan provides guidelines on priority actions to combat threats to muriquis and ensure species conservation (Jerusalinsky et al. 2011). Among the goals and actions are the quantification of remnant populations, assessment of genetic variability within and

between populations, and the establishment of an integrated program to monitor demographically the populations identified in conservation priority areas (Jerusalinsky et al. 2011, Strier et al. 2017). Despite the importance of muriqui conservation and the effort of the stakeholders, most of the northern muriqui populations still have missing or deficient data about baseline population parameters, genetic status and local threats. Most of the knowledge about *B. hypoxanthus* originates from a single population that inhabits the 957-ha of the Private Reserve Feliciano Miguel Abdala (RPPN-FMA), where one social group has been systematically studied since 1982 (Strier & Boubli, 2006).

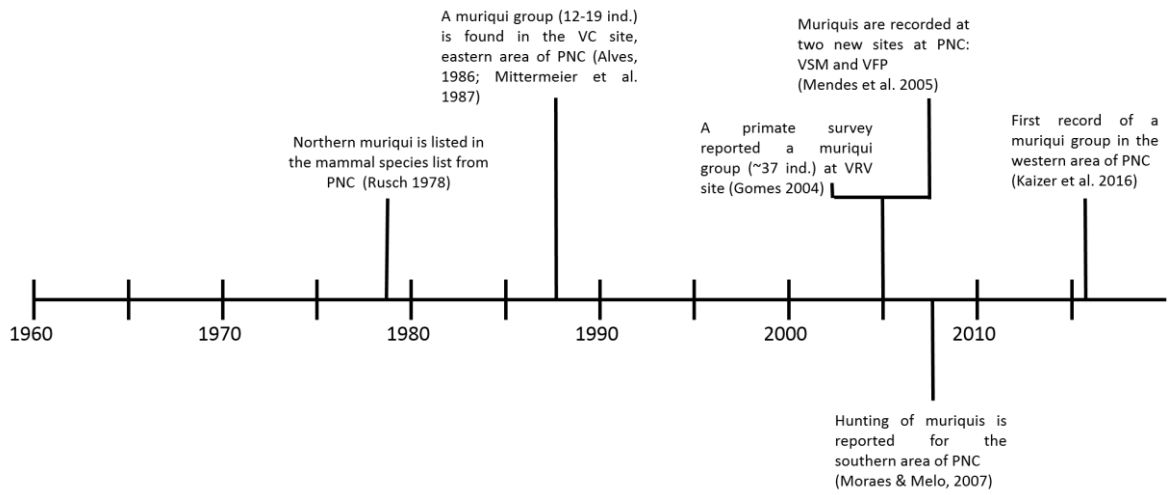
1.2 | Caparaó National Park (PNC): one of the last strongholds for northern muriqui conservation

The Caparaó National Park (PNC) consists of a federal protected area with 318.5 km^2, located on the state border of Minas Gerais and Espirito Santo, in southeastern Brazil (location number 12 in Figure 1, 20°37’ and 20°19’ S; 41°43’ and 41°55’ W). Predominantly montane, with altitude ranging from 997 to 2890 m above sea level, the PNC has a geographic importance for conservation of the muriquis as it harbours the species population inhabiting the highest altitudinal range (up to 2,000 m above sea level). With a strategic location between known muriqui populations, the PNC has the potential for the future implementation of corridors with, at least, two nearest remaining muriqui populations - the Brigadeiro State Park (PESB; apart ~55km), and the private reserve RPPN-Mata do Sossego (RPPN-MS; apart ~40km).

Being the third largest area that harbours the species, the PNC is classified as one of the four priority areas for the northern muriqui conservation in the long-term

(Melo et al. 2018). Furthermore, a recent evaluation on priority populations for the demographic monitoring of the muriquis highlited the PNC due its potential to hold large populations and the ecological uniqueness of the habitat (highest altitute of the species, Strier et al. 2017). However, despite its importance, the knowledge on this population is characterised by huge data gaps (see Box 1). Consequently, little is known about the distribution, population size (and composition), and genetic status of the muriquis at PNC. The remoteness of the forest habitat create difficulties in accessibility, and logistics are the major constrains for the assessment of northern muriqui population at PNC. In addition, muriquis groups may have large home ranges (168-309 ha), travel long daily distances (200-2,835 m; Dias & Strier 2003), and show fast arboreal movement (Strier 1992), which make the implementation of traditional ground-based surveys more difficult. Furthermore, ongoing hunting pressure makes the systematic monitoring by habituation of groups undesirable, and can be potentially deleterious to the population, despite the PNC being a protected area. For these reasons, there is an urgent need for effective monitoring tools to assess northern muriqui population and their trends over time, in the same time that are feasible to employ in remote forests and that are minimally invasive to the study population.

Box 1. Reports of Northern muriqui (*Brachyteles hypoxanthus*) at Caparaó National Park (PNC)



The figure shows the timeline of records and the gaps on the studies on northern muriqui at Caparaó National Park (PNC). The muriquis at PNC were first confirmed in the literature by Rusch (1978), who published a list of mammals and birds for the park, however, the author did not informed when, how and in which areas of the park the survey was conducted. From May-1979 to Oct-1980 a systematically survey on mammalian community across an altitudinal range was conducted by Blair (1989) in the western and northestern areas of the park. The author mentioned the possibility of species extinction in the park since no sightings of muriquis had been recorded during the systematic survey neither during the opportunistic surveys on the eastern side where the species had been reported by hunters and park guides. Several years later, Alves (1986) and Mittermeier et al. (1987) reported the occurance of a muriqui group with ~12-19 individuals in one of the valleys located in the eastern area of the park: VC, *Vale do Calçado*. A new gap of almost 20 years remained until Gomes & Melo (2005) conducted a survey on primate species at the Rio Veado Valley (VRV), on the eastern side of the park, and reported the occurrence of a muriqui group with ~37 individuals in that location. Then, Mendes et al. (2005) recorded the species in two new locations in the eastern area of the park: VSM - *Vale do Santa Marta*, and VFP - *Vale do Facão de Pedra*. An act of muriqui poaching in the south region of the park was reported by Moares & Melo (2007), but the authors only heard the vocal calls of the species. Again, a gap of approximately 10 years remained until Kaizer et al. (2016) found a new group of muriquis with approximately 40 individuals in the western area of the park (VA: *Vale do Aleixo*). This finding represented the first recorded of the species for the western area of the park, and of the highest altitude occurrence of the species (1900m).

1.3 | Canopy camera trap as an effective tool for arboreal primate assessment

Camera trapping is a non-invasive method characterized by the use of fixed autonomously triggered cameras (remote cameras or camera traps), triggered by infrared sensors, to catch images of animals passing in front of them. This non-invasive tool is equally efficient at collecting data during the day and night, under diverse climatic conditions, and in remote areas or difficult terrain where traditional methods are likely to fail (Karanth & Nichols, 1998; Silveira et al., 2003; Tan et al., 2013).

From pioneering monitoring of big cats (Karanth & Nichols, 1998; Silver et al. 2004), the use of cameras traps has increased considerably in the past 10-15 years, now addressing to monitor a diversity of taxonomic groups (*small mammals*: Palmeirim et al. 2019; De Bondi et al. 2010; Glen et al. 2013; *birds*: O'Brien & Kinnaird 2008; *reptiles*: Welbourne et al. 2017; *frogs*: Barata et al. 2017; Hobb & Breme 2017), including primates (Pebsworth & LaFleur, 2014). Recent technological advances and reduction in price, has allowed for exponential growth in the use of camera traps in wildlife studies, the number of studies doubling approximately every 3 years in the past decades (Burton et al. 2015). Adopted in thousands of published studies to date, camera traps have been applied for planet's biodiversity monitoring due their potential of standardized protocols (e.g. Beaudrot et al. 2016, Steenweg et al. 2017). A ten-year review by Burton et al. (2015) included 20,000 cameras sites demonstrating that cameras are deployed in thousands of sites across the globe, monitoring a great diversity of species. Camera trapping has proven to be an effective tool for detection and monitoring terrestrial wildlife and are now a ubiquitous tool in ecology and conservation (Wearn & Glover-Kapfer 2019; Caravaggi et al. 2017, Burton et al. 2015).

Despite the widespread adoption of camera traps and their effectiveness, the use of the camera trapping to study arboreal species in the forest canopy remains rare. The scientific research effort on mammals using camera trapping, for example, as measured by a quick search on the number of published articles on the Web of Science database up to June 2019, yielded 2931 records with the query 'camera trap*' AND 'mammal'. Whereas the same search using the query 'canopy camera trap*' AND 'mammal' generated a list of 81 records.

For arboreal primates, the use of camera trapping have been increasing in recent years, but it was only after the methodological study by Gregory et al. (2014) that camera traps emerged as a promising tool to survey arboreal species in the upper forest canopy (Bowler t al. 2017, Withworth et al. 2016, Gregory et al. 2017). To date, camera traps were used to examine fig consumption by Yakushima macaque in tree canopies in Japan (Otani, 2001). Kierulff et al., (2004) placed camera traps 2m above ground in baited-stations to survey buff-headed capuchin monkeys (*Sapajus xanthosternos*) in Atlantic Forest of Brazil. Easton et al., (2011) distributed five camera traps in a bamboo forest to survey the owl-faced monkey (*Cercopithecus hamlyni*). Olson et al., (2012) examined different arboreal camera trap techniques to document greater bamboo lemurs (*Prolemur simus*) in Madagascar. Tan et al., (2013) used camera traps to report the diel pattern of Guizhou snub-nosed monkey (*Rhinopithecus brelichi*) in China. Chen et al., (2015) used arboreal camera trap to estimate group home range and the vertical range used by the newly discovered *Rhinopithecus strykeri*. Gregory et al. (2017) placed camera traps in the natural bridges in the canopy of Peruvian Amazon to assess primate response to oil pipeline construction. Nail et al. (2019) detected the rare white-naped Mangabey *Cercocebus lunulatus* in canopy forest of Ghana.

In view of the challenges associated with observing most arboreal primates from the ground, especially species that are rare or elusive that occur in remote habitats or in areas with high hunting pressure, the use of camera traps in the canopy can circumvent these issues and provide an unbiased means of population assessment within remote and hunted areas (Whitworth et al., 2016). Similar to terrestrial camera trapping, canopy camera trapping could be a cost-effective, relatively low-effort technique, to survey simultaneously huge areas, and allow long-term continuous monitoring of a specific species of interest (Gregory et al., 2014).

1.4 | Non-invasive genetic sampling for Northern Muriqui population assessment

Genetic monitoring strategies have been developed to assess the temporal changes in population genetic metrics and other population data, using genetic markers (Scwartz et al. 2007). Genetic monitoring can be used to assess a range of parameters on wildlife populations, both demographic parameters such as population size and abundance, and occupancy, as the genetic parameters such as genetic diversity, structure and effective population size (Stetz et al. 2011, Scwartz et al. 2007, Carrol et al. 2017). Genetic approaches allow the addressing of a range of questions in the fields of ecology, behaviour, and conservation in an evolutionary context (Vigilant & Guschanski 2009).

Until recently, the genetic monitoring of wildlife populations has been limited by availability of good quality DNA samples (i.e. blood and tissue). However, technological advancements have revolutionized the assessment of wildlife

populations, especially from the use of non-invasive samples in genetic monitoring approaches (Waits et al. 2005, Beja-Pereira et al. 2009). Non-invasive samples, such as faeces, hair, urine, feathers and others are among the choices for genetic monitoring in a variety of taxa (Carrol et al. 2017), including primates (Arandjelovic & Vigilant 2017; Mcneilage et al. 2006). Non-invasive samples have the advantages to allow individual identification across space and time without the need of capture, disturb or even seeing the individuals (Beja-Pereira et al. 2009).

However, the low quality DNA from non-invasive samples may limit the application of some molecular techniques, such as modern genomics methods (e.g., RAD- seq; Carrol et al. 2017), and a range of methodological and protocols are often established to maximise success and overcome the degradation, fragmentation and errors rates when working with non-invasive samples (Beja-Pereira 2009, Nechvatal et al. 2008, Miquel et al. 2006, Wang 2016). Faecal samples (Figure 2), for example, do not always yield enough DNA and specific protocols should be followed from sample collection to data analysis. Success rate of faecal samples can be influenced by a variety of factors, such as bacterial and parasite presence, diet, time of exposition after defecation, environmental temperature, rainfall, individual health, preservation and storage methods, researcher experience, and extraction and PCR methods (Nechvatal et al. 2007, Nsubuga et al. 2004, Arandjelovic & Vigilant 2018, Arandjelovic et al. 2009, Chaves et al. 2006, Beja-Pereira et al. 2009).

The noninvasive sampling was first introduced on the genetic monitoring of brown bears (Hoss et al. 1992; Taberlet et al. 1992) and chimpanzees (Morin et al. 1992) approximately 25 years ago. Since then, researchers and conservationists have been demonstrating the importance of genetic monitoring to a variety of applications. Noninvasive genetics have been used to: detect rare species; estimate population size

and demographic parameters; evaluate social structure; movement and migration; monitor disease; quantify genetic diversity; and examine historical and contemporary genetic patterns of a species (Bruford et al. 2009).

Despite the variety of genetic approaches applied in primate studies (Vigilant & Gustanski 2009) and the continuous advances in non-invasive molecular methods (Carrol et al. 2018), there are only a few studies applying such genetic approaches to the northern muriqui. To date, noninvasive genetic sampling using faecal samples of muriqui was first evaluated only a few years ago (Chaves et al. 2006), and there are few published studies on the species (Fagundes et al. 2008, Chaves et al. 2011, Strier et al. 2011).

Figure 2. Workflow from noninvasive fecal sampling to DNA extraction in laboratory.

Aims of the PhD thesis

My thesis work proposes to apply an integrative approach of non-invasive methods (remote camera trapping and genetic assessment) to fill our gaps of knowledge on a conservation priority northern muriqui population.

Here, are enclosed three research chapters that focus on three main issues:

I) Evaluate camera trapping as an effective method in surveying arboreal primates by means of comparing the detection efficiency between camera trap *vs* traditional line transect and assessing the effect of study design on the detection probability based on the number, distance and position of the camera trap placement in the canopy.

II) Investigate whether camera trapping could be used for accurate measurements of population size and demographic composition of non-habituated primates by describing the demographic measures obtained for the studied groups and comparing the minimum group size obtained from arboreal camera trapping to the number recovered by genetic tagging.

III) Examine the genetic variation and diversity of northern muriqui populations across the majority of its range using mitochondrial DNA (mtDNA) and examine the historical and current variation and diversity within PNC populations based on mtDNA and microsatellite markers.

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Chapter II

INTO THE CANOPY: EFFICIENCY OF CAMERA TRAPPING TO ADVANCE ARBOREAL PRIMATE MONITORING IN DENSE RAINFOREST

Chapter II

Into the canopy: efficiency of camera trapping to advance arboreal primate monitoring in dense rainforest

2.1 | INTRODUCTION

Primates are facing a global extinction crisis (Estrada et al., 2017). To allocate effective conservation actions, researchers and conservationists need effective monitoring tools to assess species' populations and their changes over time (Plumptre & Cox, 2006). However, inventorying and monitoring arboreal primates are significantly challenging because of the variation in species' detectability. For example, species differ in body-size, are fast-moving and can be shy and/or elusive (Campbel et al. 2016). There are also difficulties in surveying in dense vegetation or remote forests. In addition, standard ground-based transects to survey arboreal species usually have quite large confidence intervals, lead to disturbance to animals, are commonly biased to large/ conspicuous species and by experience of the observers, and are costly and time consuming (Whitworth et al. 2016; Buckland et al. 2010; Plumptre & Cox, 2006). As a result, populational data for many arboreal species are lacking.

Recent technological advances, including camera traps (Capelle et al., 2019; Pebsworth & Lafleur, 2014), passive acoustic devices (Kalan et al., 2015), and conservation drones with thermal imaging (Spaan et al., 2019), are now leading to advances in remote wildlife monitoring. In recent years, camera trapping has revolutionized wildlife ecology and proven to be a ubiquitous tool in animal survey and

monitoring (O'Conell et al., 2011; Burton et al., 2015; Wearn & Glover-Kapfer, 2019). The versatility of camera traps allows non–invasive sampling of multiple species and sites simultaneously, gathering precise and up-to-date data about population size, distribution, habitat use, and addressing a range of ecological and behavioral questions (Caravaggi et al., 2017; O'Conell et al., 2011). However, despite the versatility of camera traps and their recent technological improvements (ranging from improved battery life and data storage through to wireless and real-time data transmission), the use of camera trapping to survey arboreal species in the rainforest canopy remains rare (Gregory et al., 2014). In the Neotropical region, the main approaches using camera traps in the high forest canopy emerged after the guidelines provided by Gregory et al. (2014), with most of the studies focusing on monitoring natural and artificial bridges over oil pipeline (Gregory et al., 2017a, 2017b; Balbuena et al., 2019). There are only a few studies that have been attempted in continuous forest, focusing on surveying arboreal mammal species richness (Bowler et al., 2017; Whitworth et al., 2016), or the effects of human disturbance on arboreal mammals (Whitworth et al., 2019).

Unlike terrestrial camera trapping where the subject is in a two-dimensional environment, detection efficiency of arboreal camera trapping can be more challenging in the three-dimensional environment of the forest canopy (Wearn & Glover-Kapfer, 2017). Detection efficiency, the likelihood of detecting a species given its presence in a surveyed site, is of high importance in wildlife monitoring since it may influence the measures of species abundance, density, occupancy and, consequently, may affect the effort and management decisions (O'Connor et al., 2017). Yet, a number of factors may also affect the detection efficiency of camera trapping (Hofmeester et al., 2019). Several studies have provided information on the influence of sampling design (e.g.

number, height, and camera position) and camera models (Hughson et al., 2010) on the detection efficiency of terrestrial camera trapping, and on their effectiveness compared to other methods (Silveira et al., 2003; Rovero & Marshall 2009). In the canopy, a methodological work by Gregory et al. (2014) showed, that false-triggers is higher in the arboreal camera trapping (98%) than in ground camera trap surveys. Whitworth et al. (2016) showed that camera trap placement in the mid-canopy (~10m) was less efficient than in the upper canopy (~30m) to survey arboreal mammals. And Bowler et al. (2017) and Whitworth et al. (2016) compared mammal species richness detected by arboreal camera trapping with line transect survey. Despite the importance and contribution of these studies to understanding the efficiency of camera trapping, studies focusing on detection efficiency of camera trapping in the three-dimensional environment of the canopy are missing and should be tested in order to improve the success of future studies and the monitoring of arboreal forest-dwelling species.

In this study, we used camera traps in the canopy of the Atlantic Forest of Brazil to evaluate the efficiency of this tool to survey arboreal primates. Our goals were three-fold: 1) to evaluate the detection efficiency of camera traps for the survey of arboreal primates in a dense rainforest compared to the traditional line transect method; 2) to assess the effect of the study design on the detection probability of the species by comparing different number of camera traps placed at different distances in a linear array; 3) to assess the influence of camera trap position (i.e. orientation) in the canopy on the detection rate of arboreal primates and on the proportion of blank events. We also provide a cost comparison between survey methods to better inform future studies.

2.2 | MATERIAL AND METHODS

Study site

The present study was conducted at Caparaó National Park (*Parque Nacional do Caparaó* - PNC), located on the state border of Minas Gerais and Espirito Santo, in southeastern Brazil (20°37' and 20°19' S; 41°43' and 41°55' W). The PNC comprises an area of 318.53 km^2. Predominantly mountainous, with altitude ranging from 997 to 2892 m above sea level (CMBio, 2015), the park is characterized by a variety of vegetation types that range from Altimontana Dense Rainforest and Semideciduous Forest in the lower altitudes, to evergreen forest and high-altitude grasslands (Rizzini, 1979). The PNC is one of the three locations in Brazil with the Cwc climate, according to the Köppen classification (Alvarez et al., 2014), with dry and cold seasons during May to September and a cool summer during October to April. The mean annual temperature is 9.4 C in the upper altitudes and annual rainfall is around 1,300 mm.

We sampled two rainforest sites within the park: one in the western area of the park, known as *Vale do Aleixo* (VA), characterized by Montane Seasonal Semideciduous forest, and one in the eastern area, known as *Vale do Santa Marta* (VSM), characterized by Montane Dense Ombrophilous forest (Fig. 1). The altitude at the sampling points ranged among 1308-1706 m and 1045-1442 m in VA and VSM sites, respectively. At least five primate species are known to occur in these sites, of which three can be found in both (northern muriqui - *Brachyteles hypoxanthus,* buffy-headed marmoset - *Callithrix flaviceps,* and the black-horned capuchin - *Sapajus nigritus*), whereas the black-fronted titi monkey (*Callicebus nigrifrons*) is restricted to

VA site and the brown howler monkey (*Alouatta guariba clamitans*) to the VSM site (Kaizer et al., 2016, Culot et al., 2019).

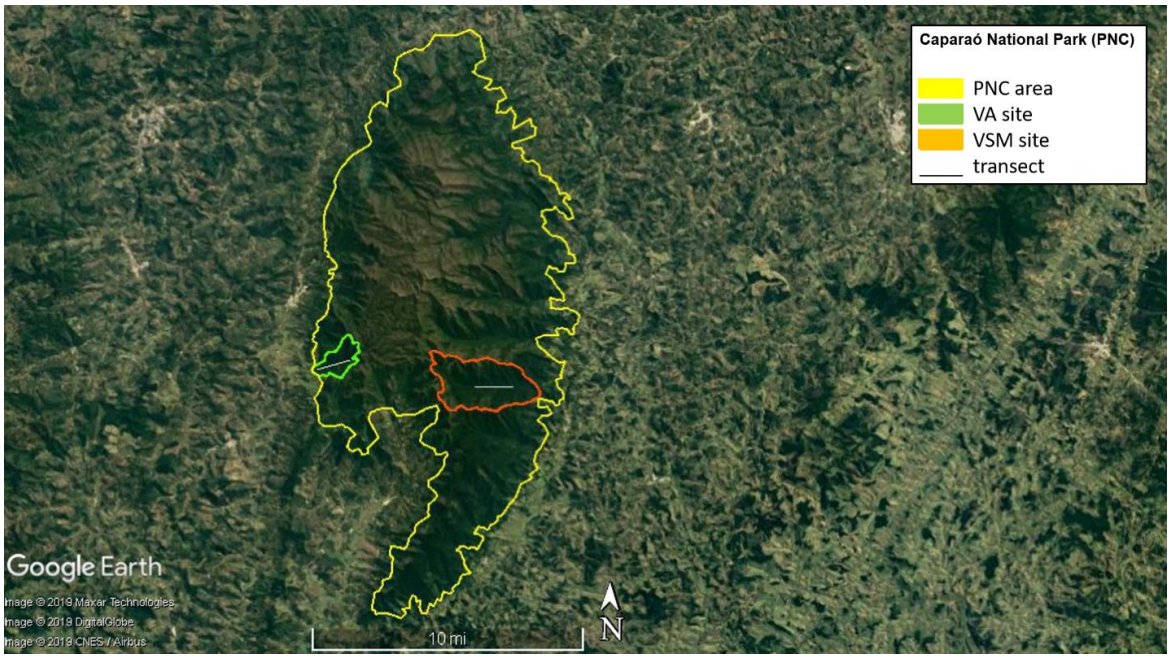

Figure 1. Map of Caparaó National Park (PNC), Brazil, including locations of the two study sites: VA (left, green) and VSM (right, orange). The 2 km transect is indicated by line.

Canopy Camera Trap Sampling

A linear array of eight cameras traps (Bushnell Trophy CamTM, #119774C; Bushnell Outdoor Products, USA) were deployed at the middle of January 2017 in the VSM site and at the beginning of February 2017 in the VA site. The arboreal sampling locations were on average 255.7 m ± 66.9 (SD) apart along a two-kilometre transect in each site. Considering the remoteness and the difficult access terrain, we used a linear array, instead of a grid, to investigate the ability of non-baited, multi-camera arrays on the detection probabilities of arboreal primates. Linear arrays were deployed longitudinally on the forest valley, along an altitudinal gradient, and parallel to the main valley stream. Cameras were set to work 24 hr/day, at a high sensitivity, and at hybrid mode - triggered to take two photographs (8MP) followed by one 30-s HD video (1280

x 720 pixels) per stimulus, and with 10 second interval between triggers. Each camera was equipped with 32 GB memory card and eight AA alkaline batteries. Over a 12-month period of survey, we visited arboreal sampling points every three months to perform camera maintenance and exchange memory cards and batteries.

Canopy Camera Trap Placement

Since the survey was conducted in a densely vegetated and continuous forest, the trees were chosen according to their safety to climb and the presence of connection between trees by the proximity of its branches forming an aerial pathway through the canopy (i.e. tree connectivity). Trees were connected to at least three other neighbour trees allowing the detection of potential arboreal mammals independently of species or animal's age. No baits or lures were used to attract animals, and no fruiting trees were chosen as places for cameras, to avoid bias data. We used rapid ascent/descent (RAD) and single rope (SRT) techniques (Maher, 2006) to climb the trees, and positioned the cameras to monitor the potential arboreal pathways to detect animals in a neighbouring tree and/or along a horizontal branch in the same tree (Fig. 2B, 2C). The mean height of the arboreal sampling location was 11 m (range = 7.5 - 16 m). We placed the camera traps on or close to the tree's trunk (to prevent movement due to the wind and to not interrupt arboreal pathways), without the use of brackets or mounts to secure the cameras (to prevent injuries to the trees and reduce costs). We removed nearby vegetation to the camera using a tree trimmer of adjustable lengths (to minimize false triggers events). We also tested all cameras and reviewed sample images during the placement, to ensure their functionality and to assess if the arboreal pathways were covered within camera's detection zone. To prevent biases and to reduce exposition to sunlight, in each site we deployed all

cameras faced to the same cardinal direction (VA = northerly direction, VSM = southerly).

Figure 2. Trees with branches forming an aerial route through the canopy. White arrow (A) indicates the potential aerial pathway where arboreal animals can travel in the canopy. Arboreal camera placement focusing (B) neighbouring tree and (C) a horizontal branch; Northern Muriqui (*Brachyteles hypoxanthus*).

Camera Trap Data Management

Two people processed camera trap data. First, one person (CLN) separated the data in files according to their category (e.g. blanks, mammals, birds, reptiles), and then a second person (MCK) reviewed all the data and imported photographs into WILD.ID software v0.9.28 (TEAM Network, www.wildlifeinsights.org/team-network), where images were coded by type (i.e. Animal, Blank, Misfire, Unidentifiable) and the species were identified and number of individuals tagged. Wild.ID has an 'easy interface' allowing the management of thousands of images and automatic extraction of EXIF and metadata from photographs, minimizing the risk of human errors during data entry (Scotson et al., 2017). Data were then exported to *csv* format, and scientific names of some mammal species were updated according to Paglia et al. (2012). As WILD.ID did not support moving images, camera trap videos were analysed in Windows Media Player adopting 1.4x speed.

Once the camera traps were set to hybrid mode, an event was defined as a set

of two photographs and one video. Events were classified as independent after an interval of 1-min. This conservative threshold was chosen based on the information provided by the videos, the ability of recognize some individual due physical features (e.g. *Brachyteles hypoxanthus*) and in recognition of the direction of travel of animals and the absence of backtracking.

Line Transect census

To evaluate the efficiency of canopy camera trapping for survey arboreal mammals, we also performed the traditional method of line transect along the two-kilometre transect on the same trail system where camera traps were placed in both sites. Each trail was walked in the morning (07:00 – 10:00) and afternoon (13:00 – 16:00) at least 5 days per month over the period of February-June 2017, overlapping with the arboreal camera trap sampling. An experienced observer (MCK) performed the survey, and recorded all medium and large-sized mammals observed during the transect sampling. A laser rangefinder (Nikon Forestry Pro) was used to measure, as accurately as possible, the distances from the line of the first individual detected from a group (Buckland et al. 2010; Hassel-Finnegan et al. 2008).

Arboreal Camera Trapping Efficiency

To evaluate the efficiency of arboreal camera traps in detecting arboreal primates and compare the detection probability between camera trapping and the traditional line transect methods, for comparison purposes we used the following detection/non-detection data:

a) Camera trapping: five one-week occasions of arboreal camera traps data that overlapped with line transect survey at each site;
b) Line transect: five one-week occasions of two-kilometre line transect data at two sites surveyed.

Since the methods differ in area covered and units of effort, and therefore, are not directly comparable, a comparison between the relative detection efficiency per sampling method-specific unit of effort between arboreal camera trapping and transect line can be conducted using occupancy models (Sales et al., 2019). Thus, we applied a single season occupancy model (Mackenzie et al., 2002) where the abovementioned method-specific surveying data were adopted as sampling occasions to construct the detection histories (MacKenzie et al., 2017). Assuming that occupancy state (occupied/non-occupied) is constant across survey period, all sampling occasions are a potentially imperfect detection of the true level of occupancy. As our goal was to compare method-specific detection probability, we used "surveying method" as an occasion-specific covariate (survey method type: camera trap, line transect) without computing any other models. Following Sales et al. (2019), accumulation curves were computed for a comparison between methods using the equation (MacKenzie & Royle, 2005):

$$p^*_{smk} = 1 - (1 - \hat{p}_{sm})^k$$

Where p^*_{smk} represents the cumulative detection probability of a species *s*, given the species *s* is present, using method *m* after sampling events *k* times based on the estimated surveying method-specific probability of detection of each species ($\hat{p}_{sm}$). Varying k from 1 to a higher number (in this study: 30 events) we determine the number of sampling events *k* required to have $p^*_{smk} \geq 0.95$. To evaluate significant difference among detection probability of each method we used a *post hoc* Tukey test,

accounting for non-normality and heteroscedasticity. We focused the analysis on three primate species (buffy-headed marmoset, black-horned capuchin, and northern muriqui) that were detected in the two sites by both methods type (canopy camera trap and transect line) and undertook the same analysis separately for each species. The analysis was performed using the *unmarked* package in R (Fiske & Chandler, 2011).

Arboreal Camera Trapping Survey Design

Number of cameras traps in an array

To assess the effect of the study design on the detection probability of arboreal camera traps for the three primate species, we used the following subset of data:

a) eight one-week occasions of arboreal camera trap data in which all cameras in the array were simultaneously active.

We computed the same analysis as described in the session above. However, since our objective here was to quantify and compare arboreal camera trapping design-specific detectability we used the "number of cameras" deployed in an array at different distances as an occasion-specific covariate (Number of cameras: eight (~250m), six (~250m), four (~500m), three (~750m), and two (~1750m)). In the same way we computed the analysis separately for the buffy-headed marmoset, black-horned capuchin, and northern muriqui.

Arboreal Camera Trapping Position

To test the effects of arboreal camera trap position-design, we used the entire dataset obtained over the 12-month period of survey. After the first canopy camera

check two arboreal sampling location were changed due dense vegetation that obstructed the camera's field range, probably, caused by storm. Therefore, our dataset includes 3 months of 8 cameras positioned to along a horizontal branch and 8 cameras positioned to a neighbouring tree, and 9 months of 6 cameras positioned to along a horizontal branch and 10 cameras positioned to a neighbouring tree. To evaluate whether the camera traps positioning in the canopy influence the detectability of the species, we compared the detection rate between cameras across and within primate species. We used a t-test with Bonferroni correction to evaluate whether the detection rate (events per sampling effort) obtained by cameras positioned to monitor a horizontal branch in the same tree was significantly greater than the detection rates obtained by cameras positioned to monitor a neighbouring tree. We also evaluate differences for the proportions of blank events (blank events/total events) obtained between the cameras positioned along horizontal branch with cameras positioned to a neighbour tree using a test for equality of proportions. Since in the canopy blank events are responsible for a high percentage of the triggers (Gregory et al., 2014) we believe they are of great importance to provide a better understanding about the survey design for improve future monitoring studies in the canopy.

Survey methods costs

We calculated the costs of each survey method, including equipment, implementation, labour and associated field costs. Costs were standardized per survey method. It took three people to initially cut the two-kilometre transects during 5 days per site (i.e. 80 hours/person). The five-month transect survey required a total of 500 person-hours for the two sites. For the canopy camera trapping, it took two people to place the cameras in the canopy during two days per site and two people to review

the cameras during one day per site. The twelve-month camera trap survey required a total of 112 person-hours for initial set-up and four reviewing sessions. For comparison purposes, we assumed independence in each survey method and calculated costs separately. However, in reality some costs were shared between surveys (for example, GPS, vehicle and fuel) for set up and two visits at the two sites. For consistency, labour costs were set at £10/hr.

2.3 | RESULTS

Overall, we obtained an effort of 2613 canopy camera days across the 16 arboreal sampling points over the period from January to December 2017 (Table S1). After six months of survey, four cameras traps failed due to malfunction, resulting in no data produced or no video recorded, and were excluded from the analysis. Arboreal camera traps gathered 832 events of vertebrates in the canopy, of which 349 events were obtained in the VA site, and 483 events in the VSM site. Vertebrate records included 14 medium and large mammals, and in addition 157 events of small mammals unidentified (rodents, opossums, and bats), 11 birds, and a single species of reptile (Table S2). Primates represented 50.7% of the vertebrate events. Of the five primates known to occur in the area, three species that vary in body sizes were detected (Fig. S1): buffy-headed marmoset (0.4kg), black-horned capuchin (3-4kg), and northern muriqui (13kg). The first record of northern muriqui, black-horned capuchin and buff-headed marmoset occurred at one, two, and six days after the arboreal camera trap placement. The northern muriqui was detected across the 8 arboreal camera trap sampling points in the VA site, and across 5 camera traps in the VSM. The black-horned capuchin was also widely detected across the camera trap

array (VA site: 5 cameras traps; VSM site: 7 cameras traps), whereas the buffy-headed marmoset was detected at a single arboreal sampling station in the VA site and at two stations in the VSM.

Arboreal Camera Trapping Efficiency

The arboreal camera traps and the traditional line transect detected the same primate species in both sites: the buffy-headed marmoset, the black-horned capuchin, and the northern muriqui. The black-fronted titi monkey was occasionally heard vocalizing twice in the VA site in March 2017. However, neither this species nor the brown howler monkey were detected by any method during the survey period.

Detection probability by each method varied among the three primate species. Detection probability was highest for the northern muriqui, followed by the black-horned capuchin and then the buff-headed marmoset, using the two methods (Table 1, Fig. 3). Arboreal cameras traps provided a higher detection efficiency than the traditional transect line for the small- and medium-sized species (Table 1, Fig. 3): arboreal cameras had the highest probability of detecting the buff-headed marmoset and the black-horned capuchin (0.30 and 0.40 respectively) than the ground-based method of line transect (0.10 and 0.30 respectively). But difference among methods were only significant for the buffy-headed marmoset (Tukey *test*: $p < 0.001$). Unlike these species, the pattern of surveying method specific efficacy differed for the northern muriqui, where the line transect method showed a detection efficiency of 0.60 compared to 0.50 by arboreal cameras traps survey (Table 1, Fig. 3).

The cumulative detection curves for each method and each species (p^*_{smk}) over 30 surveying events are shown in Figure 3. We found a significant difference among

cumulative detection curves for the buff-headed marmoset (Tukey *test*: P < 0.001), which the number of sampling events *k* required to have $p^*_{smk} \geq 0.95$ was 8 weeks for arboreal cameras, whereas it is required 27 weeks of surveying events for the line transect. For the black-horned capuchin, a number of 6 weeks and 8 weeks of sampling events are required to have $p^*_{smk} \geq 0.95$ for arboreal cameras and line transect respectively. The cumulative curve for each method for northern muriqui increased rapidly, and a number of 4 and 3 weeks of sampling events is required to have $p^*_{smk} \geq 0.95$, for arboreal cameras and transect line respectively (see also Fig. 3).

Table 1. Detection probabilities obtained for canopy camera trapping and traditional line transect method for three forest-dwelling primates in the Atlantic forest of Brazil.

Species	*Species Body Weight* [1]	Detection Probability (CI)	
		Canopy Camera trap	*Line Transect*
Buff-headed marmoset (*Callithrix flaviceps*)	0.4 kg	0.30 (0.10 – 0.62)	0.10 (0.01 – 0.47)
Black-horned capuchin (*Sapajus nigritus*)	3.0-4.0 kg	0.40 (0.16 – 0.70)	0.30 (0.10 – 0.62)
Northern muriqui (*Brachyteles hypoxanthus*)	13.0 kg	0.50 (0.22 – 0.78)	0.60 (0.30 – 0.84)

[1]Paglia et al. (2012)

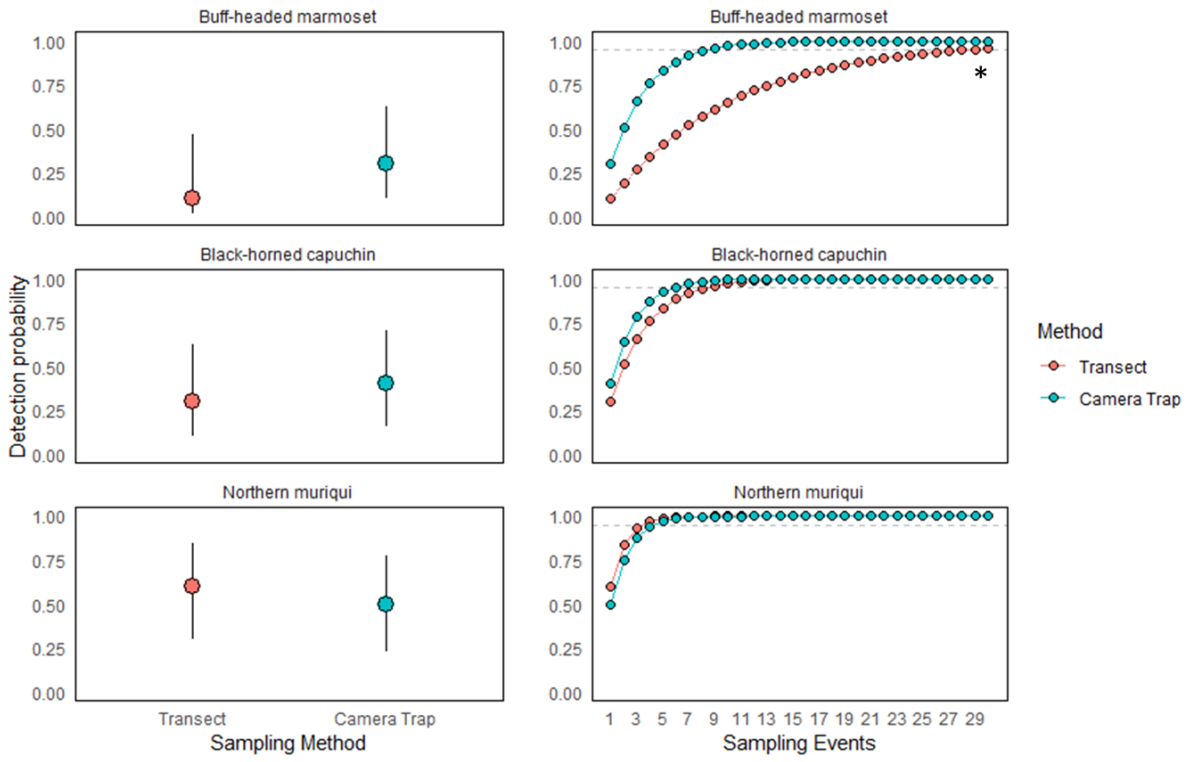

Figure 3. On the left, the detection probabilities (± confidence intervals) of each method (transect line and canopy camera trap) for the three primates' species (from top to down): buff-headed marmoset, black-horned capuchin, and northern muriqui, in Atlantic Forest, Brazil. On the right, the accumulation curves for each species indicate the number of one-week survey events for each method to reach a detection probability ≥0.95. Asterisks (*) indicate significant differences ($p < 0.05$).

Arboreal Camera Trapping Survey Design

Number of cameras traps in an array

The following patterns described here detail arboreal camera trap design-specific detectability for the three primate species. Overall, the detection probability was higher for a greater number of cameras deployed along the two-kilometre transect for all species. However, small and medium size primates, which have small and medium home range size, benefited most from an array with multiple cameras (Table 2, Fig. 4). Survey detectability was significantly lower for arrays design with cameras spaced at larger distances (≥750 m) for the buff-headed marmoset and black-horn capuchin (Tukey test: $P < 0.01$; Fig. 4). No significant differences among array designs

were found for the northern muriqui. Arboreal cameras had the highest probability of detecting the buff-headed marmoset with 4-8 cameras deployed between distance ≤500m (0.37, CI: 0.13 – 0.72). For the black-horned capuchin, array design with 8- or 6-cameras traps deployed ~250m apart had the highest probability of detecting the species (0.75 and 0.69, respectively), followed by 4-cameras spaced ~500m (0.44, CI: 0.22 – 0.68), then 3-cameras spaced ~1000m (0.25, CI: 0.10 – 0.51), and finally 2-cameras spaced ≥1750m (0.13, CI: 0.03 – 0.39). For the northern muriqui, the detection probability was highest for an array with 8- and 6-cameras traps deployed ~250m apart (0.50, CI: 0.27 – 0.73) compared to arrays with 4, 3 and 2-cameras at larger distances (0.38, 0.31, and 0.31, respectively). Yet, arrays with 8- and 6-cameras traps deployed ~250m apart showed similar detection probability (0.50), as well as arrays with 3- and 2-cameras traps deployed ~750m and ~1750m apart, respectively.

The accumulation curves shown in Figure 4 indicate the number of one-week survey events for each arboreal camera trap specific-design required to achieve $p^*_{smk} \geq 0.95$ for each species. For the buff-headed marmoset, the number of surveying events required was 6 weeks for arboreal camera trap array with 8 and 6 cameras spaced ~250m and for 4-cameras traps spaced ~500m, and an effort of more than 25 weeks is required for arboreal camera trap array with 3- and 2-cameras deployed >500m apart. The number of surveying events required for the black-horned capuchin was 2, 3, 5, 10 and 21 weeks, for an array design with 8-cameras spaced ~250m, 6-cameras ~250m, 4-cameras spaced ~500m, 3-cameras spaced ~750m and 2-cameras spaced ~1750m, respectively. For the northern muriqui, the number of sampling events required was 4 weeks for arboreal camera trap array with 8- and 6-cameras spaced ~250m, followed by 6 weeks for 4-cameras traps spaced ~500m, and

then a similar effort of 8 weeks for 3-cameras spaced ~750m and 2-cameras spaced ~1750m.

Table 2. Detection probabilities for each canopy camera trap specific-design, using a range of number of cameras traps spaced at a different distance (2-8 cameras; 250-1750 m), for three forest-dwelling primates that range in home range size in the Atlantic forest of Brazil.

Species	Species Home range size (ha) [1]	Detection Probability (CI)				
		8-Cameras traps (~250m)	6-Cameras traps (~250m)	4-Cameras traps (~500m)	3-Cameras traps (~750m)	2-Cameras traps (~1750m)
Buff-headed marmoset (*Callithrix flaviceps*)	35.5	0.37 (0.13 – 0.72)	0.37 (0.13 – 0.72)	0.37 (0.13 – 0.72)	<0.001*	<0.001*
Black-horned capuchin (*Sapajus nigritus*)	161	0.75 (0.49 – 0.90)	0.69 (0.43 – 0.86)	0.44 (0.22 – 0.68)	0.25 (0.10 – 0.51)	0.13* (0.03 – 0.39)
Northern muriqui (*Brachyteles hypoxanthus*)	309	0.50 (0.27 – 0.73)	0.50 (0.27 – 0.73)	0.38 (0.18 – 0.62)	0.31 (0.14 – 0.57)	0.31 (0.14 – 0.57)

[1]Galán-acedo et al. (2019a, 2019b)

* indicate array size with significant differences ($p < 0.05$)**.**

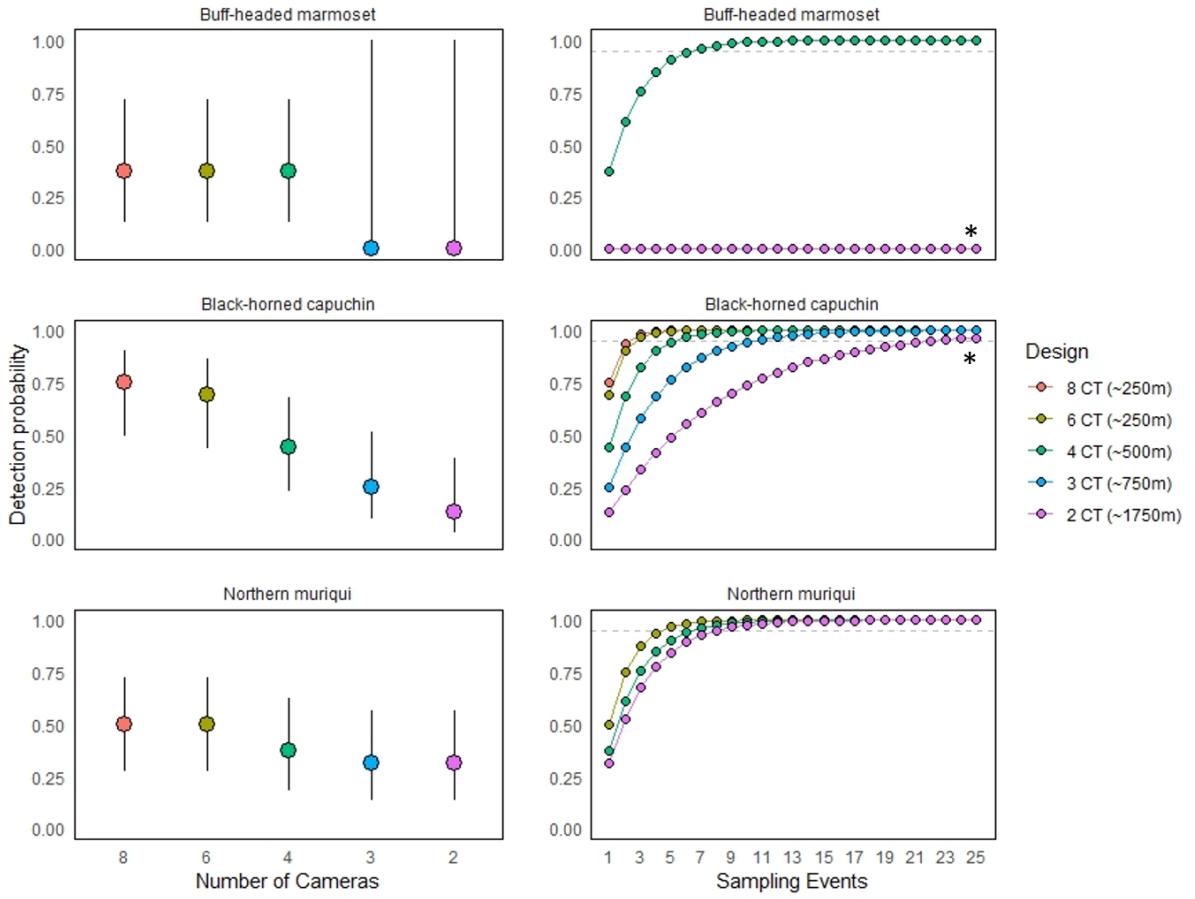

Figure 4 On the left, the detection probabilities (± confidence intervals) of each arboreal camera trap design (8-cameras traps spaced ~250m, 6-cameras spaced ~250m, 4-cameras spaced ~500m, 3-cameras spaced ~750m, and 2-cameras spaced ~1750m) for (from top to down): buff-headed marmoset, black-horned capuchin, and northern muriqui. On the right, the accumulate curve for the number of one-week survey events for each arboreal camera design to reach a detection probability ≥0.95. (CT=camera trap). Asterisks (*) indicate significant differences ($p < 0.05$).

Arboreal Camera Trapping Position

Across primate species, canopy cameras positioned to a horizontal branch gathered a total of 267 primate events while cameras positioned to a neighbour tree gathered 155 events. Due to the fact that the buffy-headed marmoset was detected only at three arboreal sampling points we tested whether detection rates (events per sampling effort) for cameras positioned to a horizontal branch was greater than detection rates obtained with cameras positioned to a neighbour tree for all primate species together and for muriquis and capuchins separately. Although detection rate

for capuchin was greater in cameras positioned along a horizontal branch than cameras positioned to a neighbouring tree (t = 1.87, df = 16, p = 0.04), no statistically difference was found after Bonferrofi correction (p adj > 0.05).The detection rates for each camera position-design across primate species can be found in Table 3.

Over the 12 months of survey period, we obtained a total of 22,275 blank events (96% of all trigger events). We found the proportion of blank events differed between the two camera positions employed. Cameras traps positioned to a horizontal branch gathered significantly fewer blank events than the cameras positioned to monitor a neighbouring tree (Chi-squared = 1162.3, df = 1, p <0.001).

Table 3. Detection rate (events/sampling effort) for each arboreal camera trap position-specific design, across and within primate species, at Caparaó National Park, Brazil, from Jan-Dec 2017.

Species	Camera Trap Position	
	Horizontal branch (*n* = 8)	Neighbour Tree (*n* = 10)
Buff-headed marmoset (*Callithrix flaviceps*)	0.01 (0.00 – 0.07)	0.003 (0.00 – 0.03)
Black-horned capuchin (*Sapajus nigritus*)	0.04 (0.00 – 0.08)	0.02 (0.00 – 0.06)
Northern muriqui (*Brachyteles hypoxanthus*)	0.16 (0.00 – 0.62)	0.09 (0.00 – 0.20)
All Primate Species	0.22 (0.03 – 0.66)	0.11 (0.02 – 0.24)

Survey methods costs

The overall costs for the 12-month of canopy camera trapping survey (£9,034.40) was lower than the total costs of five-month of line transect survey (£14,938.95). Although the initial costs with equipment purchasing for the canopy camera trapping survey was four-time higher that of the line transect survey (Table 4),

the subsequent surveys were substantially less expensive for camera trapping. The additional expense for the line transect survey was mainly due to field labour, vehicle and field stations costs.

Table 4. Total cost analysis of canopy camera trapping and traditional transect methods (within this study), to survey arboreal primates in Atlantic Forest of Brazil.

	Line Transect (GBP)	**Canopy Camera Trapping (GBP)**
Equipment		
Climbing Equipment		**£1516.65**
Cameras (16 x Bushnell Trophy Cam)		**£2756.19**
Memory Card 32GB		**£260.06**
Binoculars (Nikon Monarch)	**£399**	
GPS Garmin (+ battery charger)	**£220**	**£220**
Laser Rangefinder (Nikon Forest Pro)	**£471.95**	
Total Equipment's Cost	**£1090.95**	**£4752.90**
Implementation		
Training costs		BCAP* Climbing course **£595**
Vehicle	**£2268**	**£567** (5 month survey) **£945** (12 month survey)
Fuel	**£360**	**£180** (5 month survey) **£300** (12 month survey)
Batteries		**£289.50**
Data storage (External Hard drive 5 TB)		**£120**
Field labour for deployment	2-km transect/site (5 days x £64 x 3 people x 2 sites = **£1920**)	8-cameras array/site (2 days x £64 x 2 people x 2 sites = **£512**)
Field station costs during deployment	**£300** (10 days x £10 x 3 people)	**£80** (4 days x £10 x 2 people)
Field survey		
Field labour	**£8000** (5 month survey) (5 days x £80 x 2 people x 2 sites = **£1600/month**)	**£640** (5 month survey) **£1280** (12 month survey) (1 day x £80 x 2 people x 2 sites = **£320/visit**)
Field station costs	**£1000** (5 month survey) (10 days x £10 x 2 people x 5 month)	**£80** (5 month survey) **£160** (12 month survey) (2 days x £10 x 2 people x 2 visits)
Overall costs of survey	**£14938.95** (5 month survey)	**£7816.40** (5 month survey) **£9034.40** (12 month survey)

*BCAP: Basic Canopy Access Proficiency

2.4 | DISCUSSION

Although the effectiveness of camera traps compared with alternative surveying methods on the detectability of terrestrial wildlife are wide in the literature (Wearn & Glover-Kapfer 2019), it is still rare for arboreal camera trapping (Bowler et al., 2017, Whitworth et al., 2016). Here, we have provided evidence that arboreal camera trapping efficiency for primate species detection is comparable to or even greater than line transects in a continuous rainforest. In addition, we have presented evidence to improve the success and minimize the costs of arboreal camera trapping studies by showing the effects of arboreal camera trapping design on species detectability according the number and spacing of cameras deployed in a linear array, and the effects of camera trap position on the species detection rate and proportion of blank events.

Detection of arboreal primates using canopy camera trapping

Of the five primates species known to be occur in sites surveyed, arboreal camera traps were efficient in detecting the three species common to both sites: the critically endangered buff-head marmoset and northern muriqui (Rylands et al., 2008; Mendes et al., 2008), and the near threated black-horned capuchin (Kierulff et al., 2015). Although camera traps have a survey area limited to their detection zone, the non-detection of the black-fronted titi-monkey and the brown howler monkey by this tool in this study was probably due the absence of these species in the surveyed sites as a consequence of the yellow fever outbreak, which caused the death of thousands

primates in the Atlantic forest of Brazil between December 2016 and June 2017 (Strier et al., 2019, Bicca-Marques et al., 2017). This explanation is supported by the fact that several carcasses of howler monkeys were found in seven sites, including the VSM, within the Caparaó National Park in Feb-2017 (Kaizer, unpublished data). In addition, both titi-monkeys (Melo and Mendes, 2000) and howler monkeys (Cunha et al., 2015) commonly exchange loud calls which can be heard over long distances, and none of these calls were heard in the survey sites (titi-monkeys in the VA site: no vocal calls after March 2017). Furthermore, these primates were not detected by the transect line survey. However, it is important to highlight that a recent study demonstrated that variables such as camera height may have an influence on detection probabilities of seven arboreal mammals in Amazon forest, including spider monkey (*Ateles chamek*), saddleback tamarin (*Saguinus fuscicollis*) and large-headed capuchin (*Sapajus macrocephalus*) (Whithworth et al., 2019). Thus, the influence of canopy camera traps height from the ground in the Atlantic forest could be tested in future studies.

While wildlife-induced camera trap damage has been reported in other studies in the canopy (Gregory et al., 2014, Bowler et al., 2017), no camera was damaged by animals in this study. While the aforementioned studies used brackets and mounts to place the cameras in the canopy allowing to angle the cameras to better detect the animals, we strapped the cameras directly on the tree trunk without the use of camera mount. We believe that attaching the cameras directly on the trunk may cause less disturbance to arboreal pathways, minimizing interference on the trajectory of animals, and consequently the damage caused by them.

Comparisons between surveying methods

Only a few studies have yet compared species detection by arboreal camera trapping with traditional survey techniques in continuous forest. However, most of these focused on arboreal mammals in general and showed whether a species is or is not detected within and between the methods (Whitworth et al., 2016, Bowler et al., 2017), and only one of them provided species accumulation curves for each method (Bowler et al., 2017). In this study, we undertook a comparison of detection efficiency between arboreal camera traps and the most widely-used traditional method (line transect) for multiple species of arboreal primates in dense rainforest. Although the number of line transects and sites surveyed were limited in our study, our results are representative as the comparison between methods matched spatially and temporally, and included data from different times of the year (i.e. dry and wet season).

Arboreal camera trapping was more efficient than the traditional line transect method in detecting small and medium primates (buff-headed marmoset and black-horned capuchin) and was comparable in performance for the large arboreal primate species (northern muriqui). This result proved that camera traps are an effective tool in continuous rainforest canopy and support the notion that arboreal camera trapping has the potential to overcome the limitations of the ground-based traditional methods (Gregory et al., 2014), while traditional line transects are commonly biased towards large and conspicuous species (Bowler et al., 2017, Whitworth et al., 2016).

The information provided by the survey effort required by each method to reach a probability of detection of ≥0.95 is highly useful and may inform future studies on arboreal primates monitoring. For the small sized species (i.e. buff-headed marmoset), there is a significant difference among methods, which 8 weeks of camera trapping and 27 weeks of traditional transect lines are required to achieve the same result. For

the medium size primate (i.e. black-horned capuchin), 6 weeks of camera trapping corresponds to 8 weeks of transect lines. For the largest primate species (i.e. northern muriqui), 4 weeks of camera trapping corresponds to 3 weeks of transect lines. Thus, if a study focuses on multi-species of arboreal primate survey, 8 weeks of arboreal camera trapping would be enough to reach a probability of detection of ≥0.95 for the three species, while 27 weeks (i.e. three times more effort) of transect line is required to obtain the same result. Hence, reflecting a substantial difference in cost-efficiency between survey methods. As shown by our cost analysis (Table 4), despite the high initial cost in purchasing the climbing equipment and the cameras for the arboreal camera trap survey, the subsequent surveys were substantially less expensive than the line transect surveys. The cost of line-transect sampling represents, less expense in equipment, but costs with labour and logistics for sampling sessions were high. Therefore, for labour-intensive methods, such as the line transect surveys, double survey effort for example would double survey costs.

The quantitative evidence on the detection efficiency of arboreal camera trapping and the costs comparisons enhance the advantages of camera trapping over the traditional line transect. While at least ≥1 week of interval is recommended before to start the line transect survey after lines have been prepared (Buckland et al., 2010), our data shows that arboreal primates can be detected the next day after arboreal camera traps have been placed. In addition, line transect preparation in difficult terrains with altitude variation, such as our study site, is a difficult task and may take 5 days effort to open a single two-kilometre transect by 2 cutters and one navigator. The camera traps needed more effort to set up in the canopy (2 days/site), but an array with 8 cameras was easily reviewed by one experienced climber in only one day (40-120 min per camera). Line transects may also facilitate the entrance of illegal

poachers and increase the hunting pressure (even in protected areas; Buckland et al., 2010). All these factors should be considered, along with the spatial component and the costs required to implement each method when planning a survey and it may vary depending on the objective of the study (Whitworth et al., 2016).

It is also important to highlight that the images and videos provided by camera traps are much more informative than the raw data provided by line transect survey. Camera trapping data can unveil a range of rare events that are typically missed by the ground-based methods, especially for a poorly known habitat such as the rainforest canopy, providing considerable opportunities to address conservation-relevant behavioural topics (Caravaggi et al., 2017, Frey et al., 2017). They can also provide information of non-target species (bycatch data, see Table S2), facilitate data sharing and coordinated wildlife monitoring within the scientific community (Steenweg et al., 2017), and allow for approaches on citizen science and public awareness (McShea et al., 2016; Swanson et al. 2015, 2016). Further, a list of repositories are now available and camera trap data can be stored and act as a digital voucher museum specimens (e.g. the eMammal platform use servers from US museums, https://emammal.si.edu) and shared on the Global Biodiversity Information Facility (GBIF) platform, avoiding loss of data and guarantying its perpetuity (Cadman & González-Talaván, 2014).

Comparisons between arboreal camera trap design

Evaluating the influence of sampling design on ground camera trapping efficiency are increasingly numerous in the literature (O'Connell et al., 2011, Hamel et al., 2013, Hofmeester et al., 2019). However, empirical assessments of aspects of the

design for arboreal camera trapping applications are lacking and therefore necessary. Rather than detect a subject in a two-dimensional field, such as in ground camera trapping, arboreal species uses a three-dimensional network of pathways, which challenges the detection efficiency and may lead to failed outcomes and increased costs of surveying. As far as we know, here we have provided for the first-time evidence on the influence of the survey design (number and spacing of cameras, and camera trap position) on the efficiency of arboreal camera trapping to detect three forest-dwelling primates, which vary in body size/home range, and on the proportion of blank events produced in continuous rainforest.

Detection probability of the species was proportional to the number of arboreal camera traps deployed in a linear array, but the detection probability of small and medium size species was most influenced by the number and spacing of the cameras (Fig. 4). The number of cameras is also reflected in the survey effort required to reach a detection probability of ≥0.95. Hence, the larger the number of arboreal cameras deployed in an array in shorter distances apart required less effort than a fewer number of cameras spaced at larger distances apart. Based on these findings we could recommend an optimal number of 4-cameras traps spaced ~500m deployed in a linear array for multi-species survey studies, since the detection probability across species ranges from 0.37-0.44, and thus, considered adequate for abundance and occupancy estimates (O'Connor et al., 2017), and an effort of 6 weeks of arboreal camera trapping is required to reach a detection probability of ≥0.95 for all the species. Otherwise, for studies focusing on single arboreal primate species, the number of cameras and survey effort required vary between the species. For example, for the northern muriqui, which has large home range size, only 2-cameras spaced at ~1750m can be enough to reach a detection probability of ≥0.95 when deployed for 8 weeks, whereas, for the

buff-headed marmoset that has small home range a minimum of 4-cameras is required to have a detection probability of ≥0.95 in 6 weeks (Fig. 4). Thus, in line of terrestrial camera trapping studies, arboreal camera trapping applications might consider the body size/home range of the species, but also the ecological questions scientists wish to answer when defining the number and spacing of cameras. For inventory studies, for example, more species can be detected in less effort with a large number of cameras spaced at shorter distances. However, for occupancy studies, when multiple species are targeted, cameras should be spaced out enough to avoid spatial auto-correlation for species with larger home range sizes, but with consideration for species which may be missed with smaller home ranges (Rovero et al., 2014).

Equally as important to the number and spacing of camera traps, is how the position of the camera traps might affect detection rates of animals and the proportion of blank events produced (Hofmeester et al., 2019) in the canopy. For the northern muriqui, detection rates did not differ between the two positions of arboreal cameras traps tested in this study. However, for the black-horned capuchin detection rates were higher when the camera was positioned on a horizontal branch compared to being positioned on a neighbouring tree, despite Bonferroni correction resulted in non-significant difference. This difference may be due to the differences in body size/locomotion of the species, which have an influence on the PIR sensitivity of the cameras (Rowcliffe et al., 2011). Camera traps with passive infrared (PIR) motion sensor capture images/video in response to a rapid change in surface temperature of objects within their detection range (Welbourne et al., 2016, Apps & McNutt 2018). Thus, large animals are more likely to be detected because their large body radiates more heat than smaller ones, and as a result, can be detected at greater distances than small animals (Anile & Devillard, 2016; Hofmeester et al. 2017, Rowcliffe et al.,

2011). The northern muriqui is 3-4 times larger than the black-horned capuchin (Paglia et al., 2012), and is adapted to suspensory postures and brachiating locomotion (Hartwig, 2005), which makes them easily detectable in the camera sensitivity field in the two camera positions.

Blank events (no animals) are one of the great constraints in camera trapping studies since they can decrease the camera traps days (effort) by filling the memory card and reducing battery life. This also increases the time spent processing camera trap data (Gregory et al., 2014, Jacobs & Ausband 2018). Our results support the notion that arboreal camera trapping generates a high proportion of blank images (96%: this study; 98%: Gregory et al., 2014) and the blank events were dependent on the position of the cameras in the canopy. We have presented evidence that the proportion of blanks events is lower for cameras positioned on a horizontal branch than to cameras positioned on a neighbouring tree. This difference may be due to the difference in the "background" environment. The background surface of the cameras positioned to a neighbouring tree was more thermally heterogeneous, consisting mainly of branches, lianas and dense vegetation, which could induce more triggers due rapid change in their temperature surface when moving.

The use of camera trapping in the forest canopy is still in its infancy. Consequently, several components of the arboreal camera trapping still need to be evaluated in the future. For example, there is a need to assess the influences on detection probability of arboreal primates according to the vertical stratification of the forests, or which model of camera trap is more suitable and efficient to arboreal research. Linear array can also be expanded to a grid of arboreal camera trapping to estimate species density, territory size, and habitat use. It's clear that canopy camera trapping has great potential for monitoring arboreal primate populations and enhancing

our knowledge of the species. Since cost-efficiency of survey method is a key component when define a project, our findings demonstrated arboreal camera trapping could be a better choice to save limited resources. Future developments such as automatic processing of data (Norouzzadeh et al. 2017, Tabak et al. 2018) will make this method even more cost and time effective, potentially revolutionizing studies of arboreal primates.

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SUPPLEMENTARY MATERIAL

Table S 1. Canopy camera trapping effort at two rainforest sites at Caparaó National Park, Brazil, from January-December 2017.

	Sites		Overall
	Vale do Aleixo (VA)	Vale do Santa Marta (VSM)	
Camera trapping days	1365	1248	2613
Mean trapping days per camera	170.6 (57 – 321)	156 (19 – 323)	163.3
Cameras failure[1]	4	1	5

[1]Cameras malfunctioning after six-months of data collection

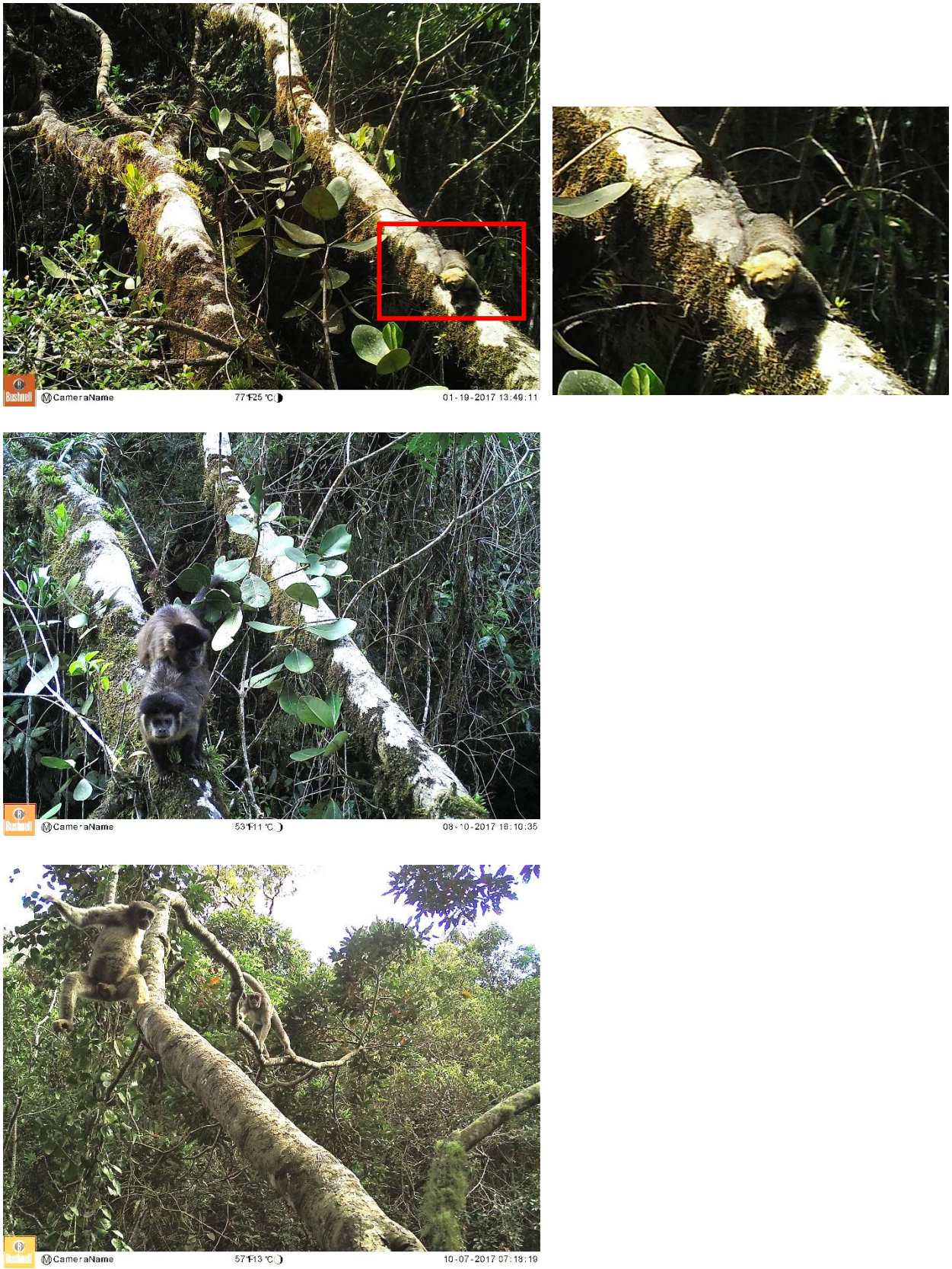

Figure S 1. Arboreal primates recorded by arboreal camera traps (from top to down): buff-headed marmoset, *Callithrix flaviceps*; a couple of black-horned capuchin, *Sapajus nigritus*; and two adult males of northern muriqui, *Brachyteles hypoxanthus*.

Table S 2. Number of events for species recorded by canopy camera traps (*n* = 16) in two Atlantic Forest sites at Caparaó National Park, Brazil, from January-December 2017.

Species			IUCN[1]	Locomotor[2]	Sites	
Order	Scientific name	Common name			VA	VSM
Mammals						
Primates	*Brachyteles hypoxanthus*	Northern muriqui	CR	Ar	200	90
	Callithrix flaviceps	Buff-headed marmoset	CR	Ar	12	25
	Sapajus nigritus	Black-horned capuchin	NT	Ar	45	50
Carnivore	*Eira barbara*	Tayra	LC	Te		19
	Leopardus guttulus	Oncilla	VU	Sc		1
	Nasua nasua	South American Coati	LC	Te		3
	Potos flavus	Kinkajou	LC	Ar		13
Pilosa	*Tamandua tetradactyla*	Southern Tamandua	LC	Sc		2
Rodentia	*Chaetomys subspinosus*	Bristle-spined Rat	VU	Ar		10
	Coendou prehensilis	Brazilian Porcupine	LC	Ar	7	
	Coendou spinosus	Spiny Tree Porcupine	LC	Ar		9
	Guerlinguetus ingrami	Southeastern Squirrel	LC	Sc		109
Didelphimorphia	*Caluromys philander*	Bare-tailed Woolly Opossum	LC	Ar	18	5
	Didelphis sp.	Opossum		Sc		2
	Philander frenatus	Southeastern Four-eyed Opossum	LC	Sc		1
	Unidentified small mammals	(bats, opossums, rodents)			24	133

Species			IUCN[1]	Locomotor[2]	Sites	
Order	Scientific name	Common name			VA	VSM
Birds						
Apodiformes	*Leucochloris albicollis*	White-throated Hummingbird	LC			1
Columbiformes	*Columba sp.*				2	
	Leptotila sp.	Dove			1	
Cuculiformes	*Piaya cayana*	Common Squirrel-cuckoo	LC		2	
Galliformes	*Penelope obscura*	Dusky-legged Guan	LC		13	3
Passeriformes	*Sittasomus griseicapillus*	Eastern Olivaceous Woodcreeper	LC			1
	Xiphorhynchus fuscus	Lesser Woodcreeper	LC		2	
Piciformes	*Ramphastos dicolorus*	Red-breasted Toucan	LC		5	1
Psittaciformes	*Pyrrhura frontalis*	Maroon-bellied Parakeet	LC		1	
Strigiformes	*Pulsatrix koeniswaldiana*	Tawny-browed Owl	LC		4	
Trogoniformes	*Trogon rufus*	Black-throated Trogon	LC			1
	Unidentified bird				9	4
Reptile						
Squamata	*Urostrophus vautieri*	Brazilian Steppe Iguana	LC		4	

[1]IUCN: CR: critically endangered, LC: least concern, NT: near threatened, VU: vulnerable

[2]Locomotor: Ar: arboreal, Sc: scansor, Te: terrestrial (Paglia et al. 2012)

Chapter III

ASSESSING GROUP SIZE AND THE DEMOGRAPHIC COMPOSITION OF UNHABITUATED NORTHERN MURIQUI (*BRACHYTELES HYPOXANTHUS*) USING NONINVASIVE BIOMONITORING

Chapter III

Assessing group size and the demographic composition of unhabituated northern muriqui (*Brachyteles hypoxanthus*) using noninvasive biomonitoring

3.1 | INTRODUCTION

Anthropogenic activities are leading to increasing threats to the world's primates. It is estimated that 75% of all primate species have declining populations, and a major extinction event may soon occur if effective conservation strategies are not implemented (Estrada et al. 2017). To access conservation status of a species, accurate information based on population size(s) and changes to these over time are required (Mace & Lande's, 1991; IUCN 2017). These assessments are also important to define effective management actions and to evaluate their success over time (Nichols & Williams 2006, Schipper & Rovero 2017).

Monitoring species populations' trends and obtaining the data needed to evaluate their conservation status depends on reliable methods that can be standardized and implemented in different locations of species occurrence (Beaudrot et al. 2016; Strier et al. 2017, Steenweg et al. 2018). For most primate species, demographic trends are typically monitored closely by direct observation of habituated groups (Clutton-Brock & Sheldon 2010, Williamson & Sheldon, 2010). However, this method is time consuming and requires trained personal, high cost investment, and may have ethical issues (Fedigan, 2010; Williamson & Sheldon, 2010). Habituation

may lead to disease transmission and increase the risks of poaching, and are not recommended in places with pressures from hunters (Strier et al. 2017; Fedigan 2010; Williamson & Sheldon, 2010). In addition, in remote forest or difficult-to-access areas, the implementation of systematic monitoring may not be feasible.

Recent technological advances in biomonitoring, such as camera trapping (McCarthey et al. 2018) and genetic tagging (Lamb et al. 2019, Palsbøll et al. 1997), may offer non-invasive alternative approaches to direct observation. Camera traps have been applied to surveying and monitoring a wide range of taxa (Burton et al. 2015), including primates (Pebsworth & Lafleur, 2014). Standardization of field protocols have also allowed for the use of camera traps in long-term monitoring of a target species in different sites simultaneously (Karanth & Nichols 2010; Steenweg et al. 2017, Scotson et al. 2017). Recently, camera trapping has been validated to survey and monitor chimpanzee communities, allowing estimates of density through distance sampling (Cappele et al. 2019), spatially explicitly capture-recapture models (Després-Einspenner et al. 2017), and assessments of demographic composition and variation (McCarthey et al. 2018). Demographic and life history data have also been obtained for wild spider monkeys (*Ateles belzebuth*) through the monitoring of a geophagy site in the forest using camera trap (Galvis et al. 2014).

Noninvasive genetic monitoring also allows for population size estimation providing an additive and complementary method for the assessment of wildlife populations (Schwartz et al. 2007; Lamb et al. 2019; Arandjelovic & Vigilant 2018). Non-invasive samples, such as fur, faeces, feathers, etc, can be used in genetic approaches to identify distinct genotypes (traditionally using a panel of microsatellite markers), which provide information on the minimum number of individuals in a given area (Waits & Paetkau, 2005; Guschanski et al. 2009; Kendall et al. 2009). Genetic

"censusing" or "tagging" can also produce a detection history of individuals that can be combined with analytical methods such as spatial capture recapture (Royle et al. 2017), allowing for an estimation of population abundance or density and its temporal and spatial variation (Miller et al. 2005; Whittington et al. 2005; McCarthy et al. 2015). In light of this efficiency to population assessment of a range of taxa (Taberlet et al. 1997; Kohn & Wayne, 1997; Palsbøll et al. 1997, Woods et al. 1999, Hájková et al. 2009; Arandjelovic et al. 2010), genetic tagging has the potential to be an effective tool for primate survey, despite it has been rarely applied in primatology (Arandjelovic & Vigilant, 2018).

To generate a genetic profile, extracted DNA samples must be genotyped at several microsatellite loci. However, this depends on how polymorphic they are. When two multilocus profiles are different, the two samples can be determined to be derived from different individuals. However, a match between two multilocus profiles does not mean that they have come from the same individual because this relies on the ability of these set of microsatellites and their associated alleles to be able to distinguish complete genotypes as unique. A common measure used is the probability of identity (PI), which is the probability that two distinct individuals sampled from a population will have equal loci or multilocus genotypes at random. PI is calculated on the assumption that individuals are not related (PI*ave*) or from siblings (PI*sibs*; Waits et al. 2001; Taberlet & Luikart, 1999). This is used to determine if the number of loci used are sufficient to estimate population size.

In this study, we evaluated the use of an integrative approach of noninvasive methods (arboreal camera trapping and genetic tagging) to obtain reliable data on population size and composition of a canopy-forest dwelling primate. The northern muriqui (*Brachyteles hypoxanthus*) is a critically endangered primate, endemic to the

Brazilian Atlantic Forest. The need to obtain reliable and up-to-date demographic data on muriqui populations was highlighted in the National Action Plan for the Conservation of Muriquis (Jerusalinsky et al. 2011). Moreover, a recent protocol has been published defining the prioritized areas and intensity for systematically demographic monitoring of wild muriqui populations (Strier et al. 2017). However, some of the areas are not feasible to implement a systematic monitoring programme and habituation may impose potential risks to the muriqui population (Strier et al. 2017). Therefore, non-invasive biomonitoring should be tested in order to improve species/population evaluations and contribute to fill the gaps in demographic data for muriqui populations.

Our aim was to test the effectiveness of arboreal camera trap to assess group size and demography composition of unhabituated muriquis. The objectives were threefold: 1) evaluate camera traps as a mean to collect demographic measures, including age-sex composition, for muriqui groups, 2) provide accurate assessment of group size based on arboreal camera trap data and genetic tagging, 3) compare the minimum group size of muriquis obtained from arboreal camera trapping with those obtained from genetic tagging, and surveys associated costs. A better understand of the aforementioned noninvasive methods to survey unhabituated muriquis in remote rainforest may inform its potential and limitations to future studies.

3.2 | MATERIAL AND METHODS

Study site

This study was carried out at Caparaó National Park (PNC), in southeastern Brazil (20°37’ and 20°19’ S; 41°43’ and 41°55’ W). The 31,853 ha protected area

comprises a chain of mountains (Caparao massif) that divides the area in two sides, east and west (Fig. 1). The landscape on the Caparaó massif includes aroboreal forest in the lower altitudes and mainly altitude grasslands above 1900 m (Rizzini, 1979). This study focused on two nonhabituated groups of northern muriqui: one recently discovered group (VA: Aleixo Group) that inhabit a forest valley of ~350 ha in the west area of the park (Kaizer et al. 2016), and another group that inhabit a valley of ~1400 ha in the east area of the park (VSM: Santa Marta Group). During the study period, only one muriqui group inhabited the west valley, known as Aleixo valley, and only one group inhabited the east valley, known as Santa Marta valley (Projeto Muriquis do Caparaó, 2019). Due the mountaineous landscape, the muriqui group in west side (VA group) did not have contact with the group in east side (VSM group) and their home ranges did not overlap. While the east valley is characterized by continuous Montane Dense Ombrophilous forest, the west valley is characterized by Montane Seasonal Semideciduous forest. The altitude ranges from 997m to 2892 m a.s.l., and the mean annual temparature is 9.4C. The annual rainfall mean is 1,300 mm, and there is one dry and cold season during May to September.

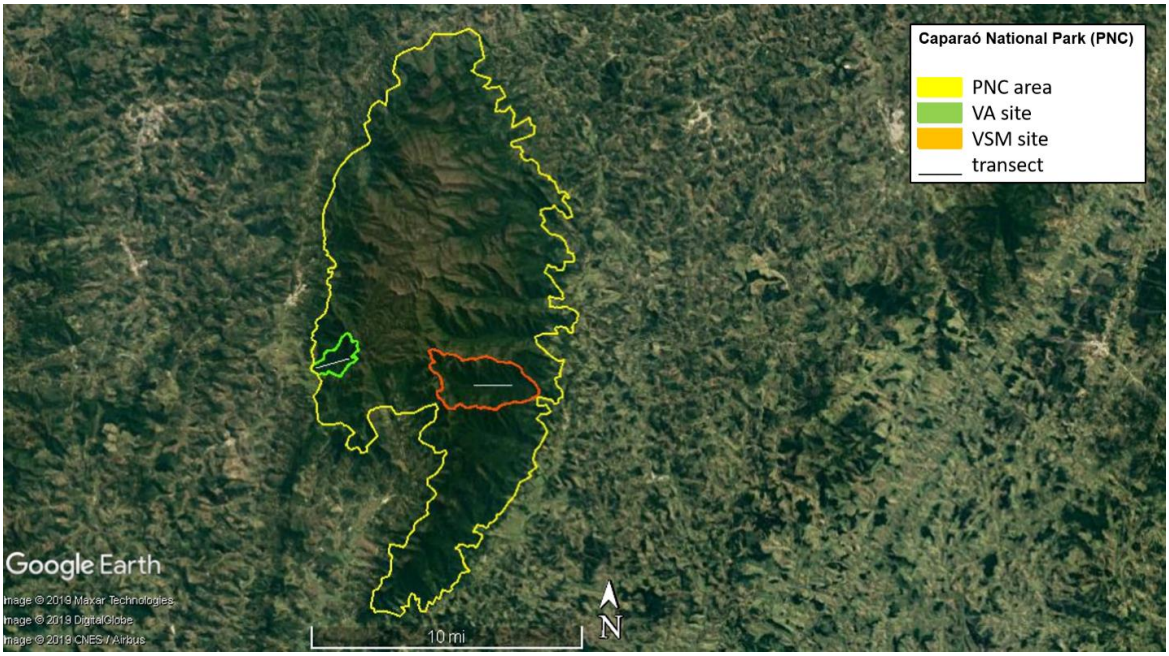

Figure 1. Map of Caparaó National Park (PNC), Brazil, including locations of the two study sites: VA (left, green) and VSM (right, orange). The 2 km transect is indicated by line.

Arboreal camera trapping

Over a 12-month period, eight camera traps (Bushnell Trophy CamTM, #119774C; Bushnell Outdoor Products, USA) were deployed in the forest canopy (mean height: 11 m, range = 7.5 - 16 m) along a 2-km linear array spaced an average 255.7 m ± 66.9 (SD) in each site. A linear array was used since it was not possible to implement a grid system due the remoteness of the areas. Each linear array was deployed across the forest valley, parallel to the mean valley stream, potentially crossing the territory range of muriquis. To ensure that groups were re-sampled in an unbiased manner (Arandjelovic et al. 2010), linear arrays covered a distance potentially no longer than the diameter of the home range of muriqui groups (Lima et al. 2019), so that the same group was sampled on different occasions. Camera traps placement in the canopy and sampling design were described in detail in Chapter 2.

A set of two pictures (8 MP) followed by a 30 s of video (HD, 1280 x 720 pixels) were obtained per trigger.

Camera trap data analysis

To investigate the group size and demographic composition of unhabituated northern muriquis population we used units of temporal events obtained by the arboreal camera trap data. The adopt of units of temporal events from camera trap data has already been used by McCarthy et al. (2018) to define party composition of habituated chimpanzees. Since we set the camera traps to hybrid mode, which is characterized by a set of still picture and a video that starts a straight after (Wearn & Glover-Kapfer 2017), we defined a subevent as a set of two photographs and one video (following Gregory et al. (2014) for a set of 3 pictures). Thus, if an individual has been captured in a picture and in the subsequent video it has been counted only once. The temporal event was defined as any subevents obtained at the same arboreal camera trap location occurring within 15 min of another on the same day (McCarthy et al., 2018). Therefore, all individuals passing in front of the same camera within the 15 min event have been counted.

Because we placed the arboreal camera traps apart an average 255.7 m along a 2-km linear array (see Chapter 2) and day travel distances of muriquis may vary from 200m to >2000m (Dias & Strier 2003), muriquis could passing in more than one camera trap in the same day. To define independence among camera traps and to avoid overestimating minimum size of muriqui groups, when muriquis were recorded in more than one camera trap in the same day, we selected for analysis that camera which recorded the largest group size. Whether the records were obtained within an

interval of 10 min of difference between one camera, we considered as independent events.

Identification of group size and composition in canopy camera data

To define the demographic composition of the population based on canopy camera traps, we counted the number of individuals classified in different age and/or sex class. Muriquis can be easily distinguish by their sex and age class due the physical features that correspond to their developmental stages (Fig. 2). Adults can reach as much as 15 kg (Aguirre, 1971), and their sex can be easily differentiated due the size and shape of their genitalia (Strier, 1987). Even for juveniles, sex can usually be determined based on the genitalia shape and positioning (Strier et al., 2006) and physical features associated to behavioural development described for muriquis may allow for identification of infants (Odália-Rimoli, 1998). A detailed description and visualization of muriquis development stages was described in a recent publication on priorities and methods for the implementation of demographic monitoring for wild populations (Strier et al., 2017).

Here, camera trap data were analysed by an experienced researcher (MCK) with identifying northern muriqui, and following the protocols provided by Strier et al. (2017) to define the age-sex class of individuals. Based on the quality of the camera trap data and associated physical features and behaviour of muriquis of a specific age class (e.g., infants are carried ventrally or dorsally by their mothers which prevent their sex identification), we extracted the number of individuals in each demographic class as follows: immatures dependent: 0-2 years; immatures independent: 2-5 years; subadults (F/M): ~5-8 years; adults (F/M): <8years (for more details, see S1 Table and

Fig. A-R in S1 File in Strier et al., 2017). The number of individuals whose age-sex class could not be defined with precision due the quality of images/video or due the position of the animal was classified as “unidentified”.

Following McCarthy et al. (2018), we inferred the population demographic composition of non-habituated muriquis as follow: i) examining the size and composition of the largest subgroup passing in a single camera trap event (i.e. within 15 min), and ii) calculating the largest number of individuals in a given age-sex class obtained in an event (e.g. the largest number of adult females detected together), then combining the maximum number of distinct individuals in each category as a proxy measure for total group composition.

Figure 2. Northern muriqui (*Brachyteles hypoxanthus*) recorded in canopy camera traps at Caparaó National Park, Brazil. From top to down: (A): two adult males on the left and one subadult in the back of the picture, (B) adult and juvenile males, (C) juvenile male, (D) adult female, (E) juvenile female.

Group size data from genetic sampling

The genetic sampling allows for a quantification of distinct genotypes from faecal/tissue samples, thus producing a minimum estimative of the number of individuals in each site (Arandjelovic & Vigilant 2017). In order to estimate group size, we compared the number of individuals obtained from canopy camera trap with the number of individuals identified by non-invasive genetic sampling. Non-invasive faecal samples were collected systematically along a pre-existence trial in each site overlapping with the camera trap period survey (over Jan-2017 to Jun-2017) and opportunistically in Jan-Feb-2018. Date, time and geographic coordinates were noted for each sample collected. To distinguish individuals from genetic sampling, we sequenced the control region mtDNA and genotyped eight nuclear microsatellite loci (Aebischer et al. 2017, Arandjelovic & Vigilant 2017; Guschanski et al. 2009; Rudicell et al. 2011; Erickson et al. 2004). The probability of identify (PI) measures for unrelated (PI_{ave}) and siblings (PI_{sibs}) were calculated in GenAlex (Peakall & Smouse, 2006). Genetic tagging of individuals was as follow:

1) Distinct individuals:

a) possessing different mtDNA haplotype and same/distinct microsatellite genotypes,
b) possessing mtDNA haplotype and differing microsatellite genotypes.

2) Same individual:

a) whether presenting matches for both genotypes and mtDNA haplotypes.

All samples identified with the same haplotype and a matching score ≥87.5% loci were re-examined for possible genotyping errors.

Since we sampled unhabituated muriquis and the genetic sampling was conducted in distinct sampling sessions, individuals could be sampled "recaptured" several times. We considered independence of genetic sampling events as follows (Arandjelovic et al. 2010; McCarthy et al. 2015):

a) distinct samples from the same individual were considered independent if were separately geographically and/or temporally in distinct days;
b) distinct samples from the same individual were considered duplicate if they were collected in the same day and a distance apart of <2800 m (based on the maximum travel distance of muriquis within one day, Dias & Strier 2003).

Survey methods costs

We calculated total purchase costs, labour expenses, data processing and on-site logistical costs associated with canopy camera trap survey and genetic tagging survey methods for our study. To increase the time- and cost-efficiency of fieldwork, collection of faecal samples for genetic tagging was carried out in parallel to other fieldwork activities, but for comparison purposes, here we assumed independence between survey methods and calculated costs separately. Labour associated with the lead author's time performing camera trapping and genetic analyses were not computed, thus we provided only costs required to data processing without include data analysis. We calculated costs for canopy camera trapping and for genetic monitoring using prices in 2018.

3.3 | RESULTS

Across an effort of 2613 canopy camera days, we obtained 948 records (pictures and videos) of northern muriqui. These records resulted in a total of 148 temporal events (15 min, Figure 3), from which 95 corresponded to VA site, and 53 corresponded to VSM site. An average of 2.45 ± 2.26 (SD, range: 1 – 10) subevents (2 pictures and 1 video), per event was obtained in VA site, and an average of 1.70 ± 0.99 (SD, range: 1 – 5) in VSM.

Camera traps detected the muriquis in 80 distinct days across the survey period, from which nine days corresponded to simultaneous records of the species in the two sites. Muriquis were recorded across the 2-km arboreal camera trap array (8-cam) in the VA site, and only in five camera traps sampling points in the VSM site. In the VA site, muriquis were recorded in canopy camera traps in 52 days, which of them 13 days corresponded to multiple records of muriquis in more than one camera (average 2.3 ± 0.5 SD, range: 2 – 3). This resulted in a range of 606.2 m ± 352 (SD, range: 255 – 1270 m). Whereas in the VSM, muriquis were recorded in more than one camera (2 cameras) in only two of those 37 days of records, over a range of 255 m.

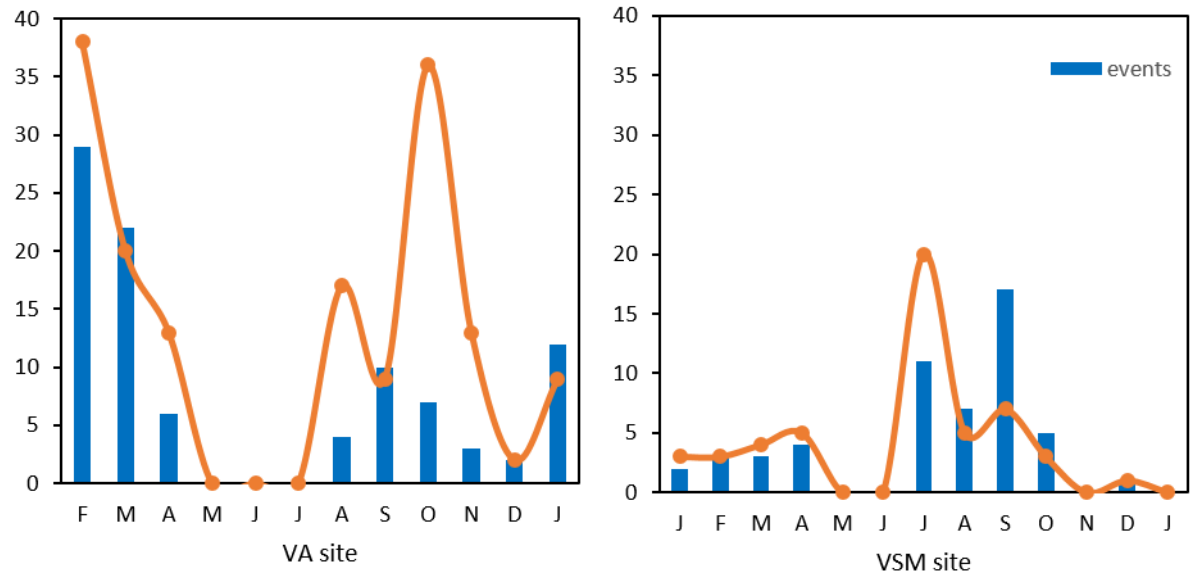

Figure 3. Seasonal variation of number of temporal events (15 min; blue bars) and maximum group-size of northern muriqui recorded in the same arboreal camera trap (orange lines), in two distinct sites within Caparaó National Park (PNC), Brazil. Survey over a 12-month period (VA: Feb-2017 to Jan-2018; VSM: Jan-2017 to Dec-2017).

Group size and demographic composition

In the VA site, the largest subgroup size captured on arboreal camera trap in a single temporal event (15 min), in October 2017, comprised 36 muriquis. Whereas in the VSM site, the largest subgroup comprised only seven individuals, in July 2017. However, when the total number of observed muriquis passing in the same camera trap in subsequent temporal events, this number increased to 20 muriquis in the VSM site and to 38 in the VA site (Fig. 3). Across all events, the summed maximum numbers of individuals seen in each demographic class estimated a number of 40 distinct muriquis in the VA site, and 23 muriquis in the VSM (Fig. 4). The maximum number of mature individuals was 21 in the VA site (M: 12, F: 6, Unidentified: 3), and 16 in the VSM site (M: 11, F: 2, Unidentified: 3; Fig. 5).

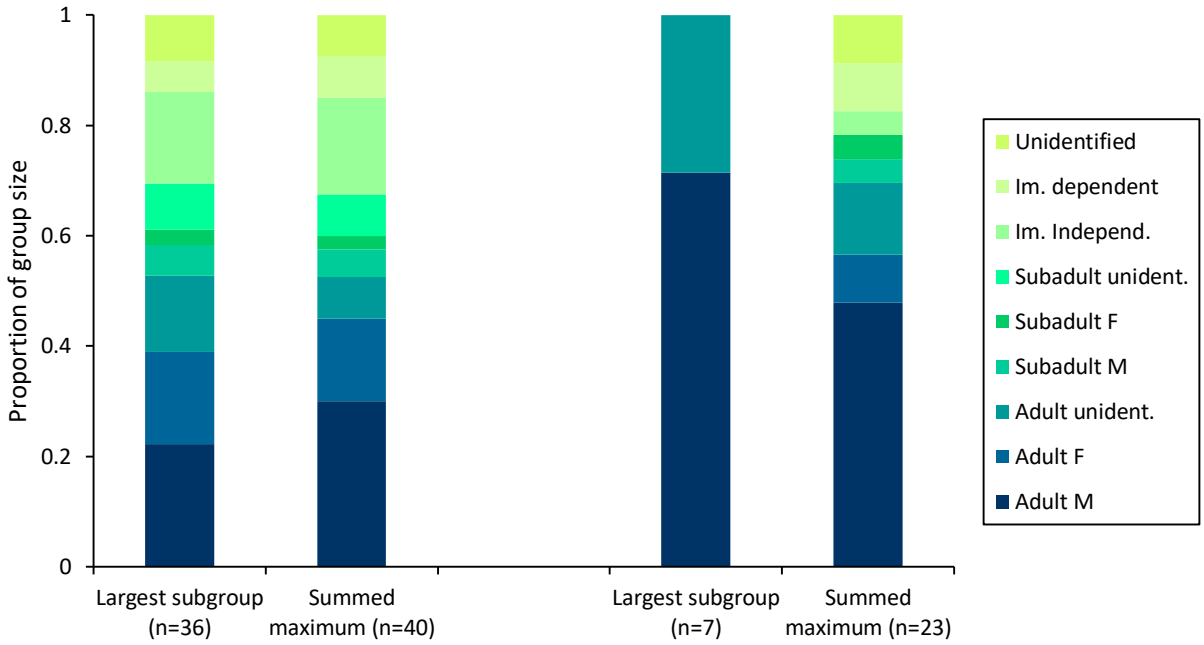

Figure 4. Demographic composition of two northern muriqui social groups at Caparaó National Park, Brazil, inferred by proxy estimates of the largest subgroup size recorded by a single arboreal camera trap event and by the summed maximum numbers of muriquis in each demographic class (Im. dependent: immature dependent; Im. Independ.: Immature independent; M: male, F: female, Unident: unidentifiable). VA group in the left and VSM in the right.

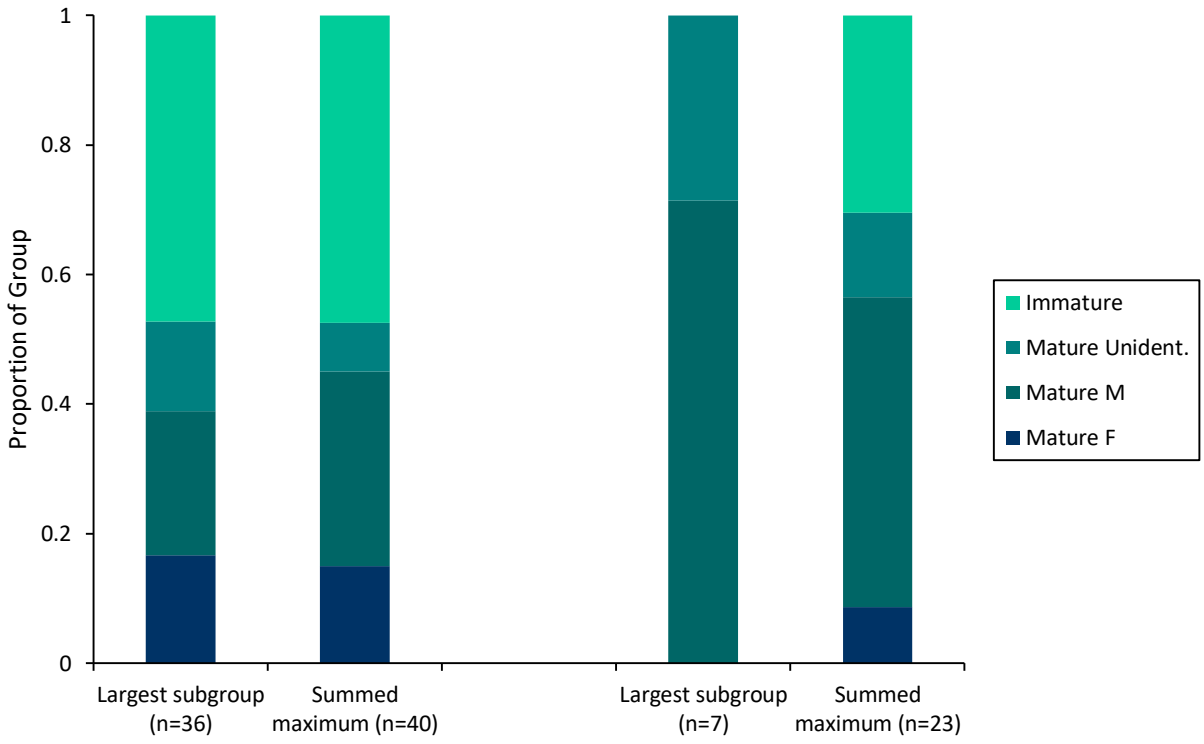

Figure 5. Proportion of mature and immature individuals in the two northern muriqui social groups at Caparaó National Park, Brazil. Measures inferred by proxy of the largest subgroup size recorded by a single arboreal camera trap event and by the summed maximum numbers of muriquis in each demographic class (M: male, F: female, Unident: unidentifiable). VA group in the left and VSM in the right.

At least three adult female were carrying dependent immature in the VA site, and two in the VSM site (Fig. 6). From these records, one represented a new born infant recorded in the VSM site, indicating that at least one birth there was in that group during the study period. The adult female was recorded carrying the infant ventrally and nursing it in July 2017, coinciding with the period of conceptions reported in other populations (Strier 2004).

Figure 6. Two distinct females of northern muriqui (*Brachyteles hypoxanthus*) with dependent infants recorded by arboreal camera trapping at Caparaó National Park, Brazil. In the left: a female was traveling through the forest canopy carrying ventrally a recent born infant in July 2017. In the right: another female was nursing the infant while resting in the forest canopy in October 2017.

Genetic tagging

Faecal samples were collected opportunistically across the period of survey resulting in a total of 60 samples. Different shape and small size of some faecal samples suggested individuals of different ages were sampled. An average of 3.83 ± 4.13 SD (range: 1 – 15) faecal sample was collected per day in 12 days in the VA site. In VSM site, the average of 2.14 ± 1.68 SD (range: 1 – 5) faecal sample was collected in seven days. Putative muriqui samples were genotyped using the eight sets of microsatellite loci and misclassified samples (n=3) were excluded. PI_{ave} across the 8 loci was 0.0000022 and 0.000038 for the VA and VSM groups, respectively and PI_{sibs} was 0.0033 and 0.013 for VA and VSM groups, respectively. Therefore, this panel of 8 microsatellite loci has sufficient power to distinguish between individuals in each of these populations. The 46 samples collected from VA group yielded in 44 usable genotypes representing 42 genetically distinct individuals. The 14 faecal samples

collected from the VSM group yielded 13 usable genotypes and resulted in 12 unique individuals.

The genotypes of the 54 resulting individuals were on average 88.62% complete. Because that few samples presented the same mtDNA haplotype and were differentiated at a minimum one loci were rechecked after genotyping matching analysis, we are confident that we did not misclassified the number of individuals.

Of those 57 usable genotypes, two of them represented recaptured individuals (VA=1, VSM=1), which were sampled in the same day separately <2800m (i.e. within the maximum travel distance of muriquis in one day, Dias & Strier 2003). In addition, one capture/recapture event was obtained in the VA site. During the 12-month period, one individual was first captured in March 2017 and recaptured in February 2018.

Canopy Camera Trapping *vs* Genetic Tagging Surveys

The genetic tagging (GT) survey provided a lower estimative of group size than that of canopy camera trapping (CCT) for the VSM group (CCT: 23 individuals; GT: 12 individuals). But group size estimates for VA group were relatively similar among both survey methods (GT: 42 individuals; CCT: 40 individuals).

The Table 6 shows the costs incurred in conducting the canopy camera trapping survey and the genetic tagging survey for group size estimation of northern muriqui in this study. The 12-month of canopy camera trapping survey costs (£9,774.40) were little lower that costs for the genetic tagging survey (£10,552.68). Our results shows that the initial equipment costs (mainly climbing equipment and cameras) for arboreal camera trapping was counter balanced by the reduced effort in field labour during data collection. The costs associated with genetic tagging

survey comprises mainly laboratory consumables and DNA processing, which are associated with the number and quality of samples. Maintaining sample collection team in field also added substantial costs to the genetic survey, which required more visits per site.

Table 1. Cost comparison between arboreal camera trap survey and genetic tagging survey for group size estimation of northern muriqui (*Brachyteles hypoxanthus*) in Atlantic Forest of Brazil. Prices are based on 2018 year.

	Descriptor	**Survey Cost (GBP)**
Canopy Camera Trapping		
Equipment	Climbing Equipment	£1516.65
	Cameras (16 x Bushnell Trophy Cam)	£2756.19
	Memory cards 32GB	£260.06
	GPS Garmin (+ battery charger)	£220
Training costs	BCAP[1] Climbing course	£595
Vehicle	Rental	£945
	Fuel	£300
Consumables	Batteries	£289.50
Data storage	External hard drive (5 TB)	£120
Field labour	Set up (8-cameras array/site)	£512)
	Review – 4 visits	£1280
Field station costs	Accommodation and food	£240
Camera trap data processing	Images and videos	£740
Overall costs of survey	**12-month survey**	**£9,774.40**
Genetic Tagging		
	GPS Garmin (+ battery charger)	£220
	Faecal sample preservation (RNAlater)	£364.80
	Field consumables (plastic tubes, gloves)	£50
Vehicle	Rental	£1323
	Fuel	£420
Field labour	Faecal sample collection – 7 visits	£2240
Field station costs	Accommodation and food	£280
DNA processing	Laboratory consumables (tips, gloves, tubes, plates, reagents, DNA kit extraction)	£3000
	Primers	£431.24
	mtDNA sequencing	£693.64
	Microsatellite genotyping[3]	£1530
Overall costs of survey		**£10,552.68**

[1]BCAP: Basic Canopy Access Proficiency;
[2]DNA processing assumes 80 samples
[3]Assuming 8 microsatellite loci

3.4 | DISCUSSION

Noninvasive monitoring such as arboreal camera trapping and genetic tagging for population assessments of arboreal primates are still underused despite both methods being used as effective tools to a range of taxa over the last 20 years (Kohn et al. 1999, O'Connell et al. 2010; Arandjelojic & Vigilant 2018, Lamb et al. 2019). In this study we provided evidence that both arboreal camera trapping and genetic tagging can be promising tools for unhabituated arboreal primates, especially for those endangered species populations where the use of systematic demographic surveys may not be feasible.

Because our study population was unhabituated and thus the real number of individuals was not known, we were not able to infer how close the findings obtained with the camera traps and genetic tagging were to the true population size. Group size estimates varied between survey methods and the two muriqui social groups. Although both methods estimates were relatively similar for the VA group, the group size estimation for the VSM group provided by the genetic tagging survey represented only 52.2% of that provided by the canopy camera trapping. This conservative estimate from genetic tagging may be a result of the difficulty to surveying infant muriquis, despite the collection of small bolus size of some samples. Usually, genetic census of primates may tend to under estimate younger individuals due the difficulties in finding small size faecal samples in the field (McCarthy et al. 2015).

In comparison between the two sites, we found the largest group size in the VA site and the largest number of individuals captured on an arboreal camera trap in a single temporal event (15 min), demonstrating potential cohesion in this group. The number of faecal samples collected together in a day also suggested similar patterns

(VA: 1 – 15; VSM: 1 - 5). It is known that flexibility in group dynamics in muriquis are common (fission-fusion and cohesive groups) and might be a reflection of the differences in demography and ecological features between the sites (Strier 2017; Strier & Mendes 2012, 2016; Silva Junior et al. 2009). Although it is outside of the scope of our objectives here, these findings suggest that arboreal camera trapping may hold promise in detecting variations in group size and their associations to habitat structure and other ecological factors (McCarthy et al., 2018).

We also found that the absence of detections of muriquis during the dry months of May and June in both sites. Muriquis is a folivore-frugivore species, and may use temporarily available resources (Strier 1991, de Carvalho et al. 2004; Tabeli et al. 2005), which might explain the absence in detections during these months. The *Mabea fistulifera* (Euphorbiacea), for example, is an important seasonal resource for muriquis in other populations (Ferrari & Strier, 1992; Mourthé et al. 2008). This pioneer species, which presents peak inflorescences in late April-May, is commonly found in the edge of forest that suffered severe disturbances or dominate the top hills that suffered forest fires (Boubli et al. 2011). Therefore, the use of arboreal camera trapping deployed in a grid or randomly distributed within the study site in future studies may contribute to our understanding of habitat use and resource distribution in remote areas.

The IUCN Standards and Petitions Subcommitee defines population size and its trends over time by means of the number of mature individuals and its variation in a population/species (IUCN 2017). Our findings revealed that both arboreal camera trapping and genetic tagging are potential tools to estimate the minimum population size of unhabituated muriquis, and arboreal cameras trap may provide the minimum number of individuals per demographic class. Furthermore, although arboreal camera trap data required some time investment to confidently classify the demographic class

of individuals, the identification of mature individuals was relatively easy and did not require individual identity recognition. Arboreal camera trapping also allowed for the identification of immature dependents, providing inference of the number of reproductive females in the population. Additionally, despite the assumption of demographic closure required by estimate population methods (capture-recapture, Karanth & Nichols, 1998; Lukacs, & Burnham, 2005), it registered birth events. Furthermore, a recent study assessing the efficiency of camera traps for surveying demographic composition and variation in chimpanzees suggested the feasibility of the method to survey unhabituated populations (McCarthy et al. 2018). By matching data on party size and demographic composition among camera trap data and direct observation of habituated chimpanzees, camera trap data tended to underestimate party size, but demonstrated proxy patterns of demographic composition (age/sex proportions) in the absence of individual identification (McCarthy et al. 2018).

The genotyping success (88.6%) based on faecal samples reported in our study was consistent to the genotyping success reported for unhabituated primate populations (gorillas: 82-95%, Arandjelovic et al. 2010, 2015; chimpanzees: 77-83%, McCarthy et al. 2015; Moore & Vigilant et al. 2014). Despite the influence of a wide range of factors (e.g. time exposed in the environment, temperature, UV, microbial activity; Beja-Pereira et al. 2009; Waits & Paetkau 2005), the genotyping success rate found in our study demonstrated the potential application of the genetic tagging to assess canopy forest-dwelling primate populations in remote areas, even for opportunistically faecal sampling. Our results revealed a conservative estimate (minimum group size) of 42 individuals in VA and 12 individuals in VSM and identified three recapture events. Timing and locations of the faecal samples were key components to identify and understand recaptures. Two of these recapture events

were collected on the same day within a very short distance; probably because of the opportunistic sampling combined with the arboreal behaviour of muriquis (i.e. faecal sample can be fragmented in distinct pieces when reached the ground). Whereas, the other recapture event indicated a spatial distance of ~300m but a temporal interval of 11 months, demonstrating that genetic tagging can also inform how this species uses its habitat. However, our sampling was conducted opportunistically because of this, and by following a linear trail limiting the number and distribution of samples obtained and, thus preventing further detailed analyses such as mark-recapture analysis, due to the lack of 'recaptures'. We suggest for future studies that defined grids should be considered since muriquis usually present large home range sizes (309 ha, Dias & Strier 2003) and move heterogeneously throughout the forest. A more systematic and consistence effort should take place instead of applying an opportunistic sampling as it may increase the sampling efficiency minimising the time effort and reducing costs (Arandjelovic & Vigilant 2018). Additionally, precision and accuracy of population size estimation increase with the number of repeated samples (Arandjelovic & Vigilant 2018). Thus, 3-4 times the number of samples compared to the number of expected/potential individuals in the study population could be collected (McCarthey et al. 2015; Petit & Valiere 2006; Arandjelovic, 2010). As a good starting point, a genetic monitoring programme might be implemented in a muriqui population of known size to determine the optimal sampling strategy and to validate capture-recapture methods to infer a better rule-of-thumb number.

Despite the limitations of our study design and opportunistic sampling and the challenge to compare surveys when the true number of group size is not precisely known, our results demonstrated that genetic samples collected over 12 days resulted in a relatively close group size estimation to that of 12-month of arboreal camera

trapping, at least for one of the groups. This finding strengthens our suggestion that a systematic genetic sampling study may be also a cost-effective tool for population size assessments of unhabituated muriquis, especially in rapid assessments (Hedges et al. 2013). Several studies have reported the efficiency of genetic tagging over traditional direct monitoring (Chancellor et al. 2012; Granjon et al. 2017; McCarthey et al. 2015) and other methods (Hádjkova et al. 2009; Arandjelovic et al. 2010; Zhan et al. 2006), suggesting that genetic counts may provide more precise and accurate counts (Solber et al. 2006). Moreover, genetic tagging can also inform sex (Di Fiore, 2005; Waits & Paetkau, 2005; Caniglia et al. 2013), age class (Carroll et al. 2018, Cattet et al. 2018), and reproductive status of individuals (Cattet et al. 2017), although these analyses have not been possible to be applied in this study due time and financial constrains.

When looking for save resources, our cost comparisons show that despite the high upfront costs of equipment purchasing to implement the canopy camera trapping survey, the subsequent sampling efforts by this method are substantially less expensive. Therefore, indicating a better cost-effective survey especially for long-term monitoring programmes (Burgar et al. 2018). Whereas non-invasive genetic tagging does not require expensive equipment to data collection, costs of laboratory consumables and genetic data processing are substantially high and will vary according to the numbers of samples and/or markers. Thus, studies that require repeated sampling sessions to collect a very large number of samples may become cost prohibitive (Hedges et al. 2013). However, genetic tagging survey may have reduced costs when combined with other field-based methods by sharing logistic effort and additional costs (Guschanski et al. 2009). Samples of good quality may also

decrease the costs of genetic analysis by minimizing genotyping errors and improving data quality (Beja-Pereira et al. 2009).

Here we revealed for the first-time evidence on the minimum population size estimate and demographic composition of two unhabituated muriquis groups from a high priority population for species conservation. Our findings suggest the feasibility of arboreal camera trapping and genetic tagging methods to fill the critical gaps in the extant muriqui populations, especially in those areas of geographic importance (Strier et al. 2017), or where systematic demographic monitoring are undesirable or poses deleterious risks to the population (i.e. hunting pressures). Although both methods have intrinsic limitations (ex. initial cost investment, time consuming of data analysis, low sampling effort), the combined application of these noninvasive approaches may offer a range of opportunities to improve wild population monitoring, not only for the muriquis but also for other arboreal primates. Finally, we observed that the total number of mature individuals revealed by arboreal camera trapping in our study groups was <50 mature individuals, which imply risks to its persistence in the long term (IUCN, 2017). They also differed greatly among sites, with the largest area presenting the smaller group size. These empirical results highlight the importance to expand our surveys to other sites within the park in order to provide a more realistic estimate of the entire muriqui population, and also to inform conservation management strategies in this protected area.

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Chapter IV

GENETIC VARIATION OF THE CRITICALLY ENDANGERED MURIQUI IN THE ATLANTIC FOREST OF BRAZIL, WITH A DETAILED FOCUS ON THE POPULATION IN CAPARAÕ NATIONAL PARK

Chapter IV

Genetic variation of the critically endangered muriqui in the Atlantic Forest of Brazil, with a detailed focus on the population in Caparaó National Park

4.1 | INTRODUCTION

The Atlantic Forest is recognized as one of the biodiversity hotspots and also one of the most vulnerable biomes on Earth (Myers 2000, Ribeiro et al. 2012). Once a vast tropical forest biome (~1.5 million km^2) it is has been drastically reduced to only 11.7% of its original vegetation cover in the last five centuries (Ribeiro et al. 2012). With most of the remaining forest highly disturbed and smaller than 50 ha, population decline and defaunation of medium to large-bodied mammals in Atlantic forest have been documented at an unprecedented rate (Canale et al. 2012, Galetti 2017).

The northern muriqui (*Brachtyeles hypoxanthus*), together with the closely related southern species (southern muriqui, *B. arachnoides*), are the largest arboreal primates endemic to the Brazil's Atlantic Forest. Approximately 400,000 muriquis were estimated to occur throughout the central-south domain of Atlantic Forest, prior the colonization of Europeans in 1500 (Aguirre, 1971). The historical deforestation and fragmentation of Atlantic forest lead to shrinking ranges and to a drastic decline of muriqui populations. Currently, less than 900 northern muriqui are known to occur in a dozen forest fragments that vary in both population and fragment sizes (Strier et al. 2017). In practice, Atlantic forest fragments with northern muriqui populations remain isolated in a human-dominated landscape coexisting with a range of secondary

anthropogenic pressures, including hunting, selective logging, forest fires, and disease (e.g. yellow fever, Strier et al. 2019; Estrada et al. 2018, Bogoni et al. 2018).

Genetic diversity is known to play an important role in the maintenance of populations' ability of successfully respond and adapt to environmental changes (Allendorf et al. 2012). In light of the intense population decline that northern muriqui have been facing in the current Anthropocene era, an assessment of the genetic structure of remnant populations is crucial to understand how the species will respond to impact of ongoing and future events and to define appropriate conservation and management measures and strategies (Frankham 2010; Frankham et al. 2010). The importance to assess the genetic status of remnant muriqui populations was highlighted in the Brazilian National Action Plan for the Conservation of Muriquis (Jerusalinsky et al. 2011, Strier et al. 2017). Nonetheless, there are limited data on genetic diversity and structure within and among northern muriqui populations (Strier et al. 2017). Only a single study has assessed the control region of 152 individuals from eight muriqui populations (Chaves et al. 2011). However, the effort sampling between populations was not homogenous and the PNC (Caparaó National Park), which is one of the four-priority areas for the species conservation in the long term (Melo et al. 2018) was represented by only two samples.

Herein, we assess the genetic diversity and structure of 11 populations of muriquis based on new samples from the PNC and from published sequences available in GenBank. The aims of our study were: 1) to quantify the genetic variability and structure by using the control region (mtDNA) sequences obtained for the muriqui populations in a wider scale by including all populations and 2) describe the patterns of genetic variability in a local scale by using mtDNA and microsatellite markers for the high priority muriqui population located at Caparaó National Park (PNC).

4.2 | MATERIAL AND METHODS

Ethical Statements

Data collection was conducted with permission of Instituto Chico Mendes de Conservação da Biodiversidade (ICMBio/SISBIO No: 54795, 49062), and Instituto Brasileiro do Meio Ambiente e dos Recursos Naturais Renováveis (IBAMA)/ Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES). In accordance with Nagoya Protocol, Genetic Heritage Management Council (CGEN) and all institutional animal care and use guidelines of Great Britain and Brazilian regulations, and University of Salford ethical approval (STR1718-14).

Sampling and DNA extraction

Muriqui faecal samples were collected opportunistically from December 2016 to June 2017 and from February to March 2018 during fieldwork activities at Caparaó National Park (PNC, 20°37’ and 20°19’ S; 41°43’ and 41°55’ W). Samples were collected noninvasively in five different sites within the park, potentially representing five distinct unhabituated groups. One additional faecal sample was obtained from a female isolated in a small forest fragment located approximately 2 km from the PNC’s border. No animals were manipulated nor disturbed during the sampling procedures. Fresh samples were collected by experienced researchers and approximately 4 ml of stool were mixed with an equal volume of nucleic acid stabilization buffer (RNALater, Ambion).

Additionally, two tissue samples were obtained: one from a subadult muriqui female found dead at 2982 m of altitude, and one from an adult muriqui male dead by electrocution in a power line near a forest fragment in the surround of the park. Both faecal and tissue samples were stored at −20°C before DNA extraction.

Total genomic DNA was extracted from tissue samples using the E.Z.N.A. Tissue DNA extraction kit (Omega) according to manufacturer's instructions. DNA from faecal samples was extracted using the QIAamp Fast DNA Stool Mini Kit (Qiagen) following the human DNA analysis protocol, with minor procedures modifications: (i) ~250 uL of the faecal slurry were added to 1 ml InhibitEX buffer, shaking for 5 min at 20 Hz of speed using Tissue Lyser II (Quiagen), before proceeding to step 3; (ii) in step 14 we added 100 uL of Buffer ATE instead of 200 uL to improve DNA concentration. Each DNA extraction was quantified using a NanoDrop 2000 Spectrophotometer (Thermo Scientific™, see Table S1).

Control region (mtDNA)

Amplification

We amplified and sequenced a 460 bp hypervariable segment of the mtDNA control region (HVRI). The PCR was conducted using the species-specific 5'-CTACTCCCTGAATAACCAAC-3' forward primer (Mono1) and 5'-AGCGAGAAGAGCGGCAAATG-3' reverse primer (Mono2) (Fagundes et al., 2008). PCR reactions were carried out in a final volume of 20 uL containing 2 uL of DNA extract, 0.5 uL of each primer, and 17 uL of MyTaq Red Mix (Bioline). Amplifications were performed at 95ºC for 2 min, followed by 35 cycles of 92ºC for 60 s, 48ºC for 30 s and 72ºC for 45 s, with a final extension at 72ºC for 5 min. The PCR products were

quantified using 1.5% of TBE agarose gels with HyperLadder 50bp (Bioline). The PCR product from tissue samples were used as a positive control, and negative PCR controls (without DNA template) were included to check for the presence of possible contaminations. Standard Sanger sequencing of both DNA strands was conducted externally by a commercial service (Macrogen Inc., Korea).

Analysis

Obtained sequences were checked and assembled in DNA Baser Assembler v5.15 (2014, Heracle BioSoft, www.DnaBaser.com). A BLAST search was conducted in the NCBI database in order to confirm the origin of the samples as belonging to northern muriqui. Additionally, previously published sequences from distinct populations already available in the GenBank database were added in our dataset for comparative purposes in a wider scale. From all the sequences of northern muriqui control region (mtDNA) available in GenBank, we excluded those with ambiguous base calls (Ns) to avoid haplotype inflation in the genetic analyses. Thus, a total of 153 additional sequences were used in downstream analyses from 10 muriqui populations (Fig. 1). Additional sequences referred to 20 sequences already published for control region of northern muriqui (Chaves et al., 2011), plus 102 unpublished sequences available in Genbank (Chaves, unpublished; see Table S2 for details of populations and GenBank accession number). Sequences were aligned using the CLUSTAL W and visually inspected in MEGA version X (Kumar et al. 2018), using the *Brachyteles hypoxanthus* sequence from GenBank (AF213966) as a reference. Poorly aligned positions and indels were removed and larger segments (442 bp) were reduced to 366 bp to overlap with the data from Chaves et al. (2011).

For all muriqui population-level analyses of diversity, we used only those populations with more than 10 individuals (Table 1). Genetic diversity based on the control region of mtDNA was assessed by the indices of haplotype diversity (*h*) and nucleotide diversity (π) (Nei 1987, Nei & Li 1979) using the software DnaSP v6 (Rozas et al. 2017).

Haplotype networks at a population and local-level were constructed using the median-joining algorithm in PopART v1.7 (Bandelt et al., 1999). In order to assess the genetic structure and differentiation in a wider scale, we conducted a pairwise F'_{ST} test (Slatkin, 1995) across PNC and 5 distinct populations, which have ≥ 10 individuals sampled. Pairwise F'_{ST} test was performed in Arlequin v3.5.2.2 (Excoffier & Lischer, 2010), with 10,000 permutations. Once we sampled different social groups at PNC, we also conducted a pairwise F'_{ST} test at a local-level to assess the genetic similarity between social groups. Furthermore, we performed an analysis of molecular variance (AMOVA, Excoffier et al. 1992) to examine the genetic structure at a local-level and the genetic distances between the groups. Because a mountain chain (Caparaó Massif) stands longitudinally within the PNC dividing the park in two main areas, east and west (and might act as a physical barrier to the muriqui dispersion and consequently to the gene flow), we tested for variation among east and west subpopulations (F_{CT}), among social groups within each subpopulation (F_{SC}), and within social groups among subpopulations (F_{ST}).

The relationship between the genetic differentiation at a population-level due isolation by distance was assessed with a Mantel test (Mantel, 1967) in R using the packages *vegan* (Oksanen et al., 2013) and *adegenet* (Jombart, 2008). Matrices of genetic (F'_{ST}) and geographic (km, shortest straight-line) distances were assessed for correlations. Geographic distances were measured between all muriqui populations

using the program Geographic Distance Matrix Generator v1.2.3 (Ersts,P.J., internet). Statistical significance was determined at the 95% confidence interval (95% CI) with 999 permutations. To assess the local-level genetic differentiation in PNC due to isolation by distance, we performed the same analysis aforementioned by using the genetic (F_{ST} values) and geographic distances (km) between each social group within PNC.

Microsatellite markers

Based on the variability and efficacy results provided in a previous study using 17 microsatellite marker loci suitable for the northern muriqui (Strier et al. 2011), a set of 8 loci was then assessed for amplification success in our study population. All faecal samples and one tissue sample were amplified for each of the 8 loci through a combination of two and three primers labelled with fluorescent dye (forward sequence) in a multiplex PCR reaction (see Table S3 for more details). The Multiplex PCR were performed in 20 uL reactions, containing 2 uL of DNA extract, 0.4 uL of each primer (10 uM), 10 uL of Multiplex PCR Master Mix (Qiagen), and 5.6-6.4 uL of H_2O (depending on the number of primer combinations used). Negative PCR blanks (i.e. without DNA template) were used as a control for each multiplex reaction. Thermal cycling conditions for each PCR multiplex were as follow: initial denaturation at 95ºC for 15 min, followed by 34 cycles of 94ºC for 30 s, 55ºC for 90 s and 72ºC for 60 s, with a final extension at 60ºC for 30 min. Automated capillary analysis were conducted on an ABI 3730XL DNA Analyser using GeneScan 400HD size standard marker (Applied Biosystems) for allele size estimates (Macrogen Inc., Korea). All samples were

replicated at least three times for all the 8 loci to avoid artefacts and minimize genotyping errors due allele dropout and the low quality of DNA in faecal samples.

Automated allele calling was conducted in GeneMapper v.4.1 (Applied Biosystems) and further visually checked for genotype determination. Following Stojak et al. (2016), genotypes were independently determined twice (by myself and N.G. Sales) for all samples per each replicate. Homozygote genotypes were accepted if they were scored on all replicates (or two of the three when one of the repeats did not amplified). Heterozygote was accepted if scored on two out of three replicates. If none of these criteria were met, alleles were classified as missing data (Stojak et al. 2016).

Analysis

First, errors in the genotyping data including scoring errors, allelic dropout and null alleles were checked in Micro-Checker v2.2.3 (van Oosterhout et al., 2004); and were all absent in the genotyping data. As the samples were collected through opportunistic sampling, some individuals might have contributed to more than one sample each. To check for duplicates or genetically identical samples, we searched for matching genotypes using Microsatellite Toolkit (Park, 2001). The following framework was applied to determine matching genotypes: i) all samples with a score match of ≥ 87.5% were selected for pairwise comparisons (value established according to the genetic differentiation obtained for the eight loci herein used through the previously published data - Strier et al., 2011); ii) matching samples were checked for the occurrence of different haplotypes; iii) samples containing matches for both genotypes and mtDNA haplotypes were assigned as putatively belonging to the same individual and only one sample was kept for downstream analyses; iv) samples with matching genotypes but showing distinct haplotypes were all included in further

analyses. As a result, we excluded four samples containing missing data (>25% of the 8-loci) and three other samples, which were potentially representing duplicates.

Estimation of the genetic diversity for the muriqui population at PNC using the microsatellite loci was measured by calculating the observed and expected heterozygosity (H_O and H_E, respectively) and the number of alleles per locus in GenAlEx v6.5 (Peakall & Smouse, 2012). Inbreeding coefficient (F_{IS}) was also estimated for each locus and each social group, with significance levels calculated by randomly allele distribution among individuals within each social group. Inbreeding coefficient (F_{IS}) and allele richness (AR; calculated only on groups with at least 7 individuals) were calculated in FSTAT v.2.9.4 (Goudet, 1995). Further, deviations for Hardy-Weinberg equilibrium (HWE) were then determined using 1000 simulations. The genetic structure and differentiation across PNC social groups at microsatellite loci was quantified for pairwise F'_{ST} (Weir and Cockerham 1984) in FSTAT, with significance determined using 1000 simulations. Isolation by distance was calculated as aforementioned in the mtDNA session applying the Mantel test using pairwise F'_{ST} values (Meirmans & Hedrick, 2011) and geographic distance between social groups.

In order to assess the current genetic cluster within PNC population, a Bayesian analyses was implemented in the STRUCTURE software V. 2.3.4 (Pritchard et al., 2000) using all individuals. A series of five independent runs (K ranging from 1 to 6 to coincide with the number of putative social groups), was implemented under the admixture model with correlated frequencies, and 180,000 MCMC repetitions following a 20,000 burn-in generations. The best fit model (K value) for the data was established by the Evanno method (L'(K), |L"(K)|, ΔK; Evanno et al. 2005) implemented in STRUCTURE HARVESTER (Earl and vonHoldt 2012).

4.3 | RESULTS

From the 79 faecal samples collected in the PNC, 77 (98%) were successfully amplified for the HVRI segment ranging between 366 to 442 bp. A total of six samples was removed from the dataset prior to further analyses. These were three samples potentially representing repeat sample from the same individual (according to the data provided by the eight genotyped microsatellite loci analyses and identified from samples containing the same haplotype) and three other samples which were genotyped for only 6 of those 8-microsatellite loci. As a result, 73 unique muriqui individuals from PNC were used in the mtDNA analysis microsatellite analysis.

Population-level of genetic diversity and structure

The mitochondrial control region sequences from 11 muriqui populations analysed in this study resulted in 26 haplotypes, five of them unique for the recently described muriqui population at PNC (Table 1, Figure 1). Haplotypes were resolved based on 23 segregating sites (Table 1). The overall haplotype diversity was high (h=0.928), and the haplotype diversity from those population with ≥10 individuals ranged from 0.00 (RPPN-MS) to 0.836 (PNC, which harbours the highest haplotype diversity within the populations with ≥10 individuals sampled). The overall nucleotide diversity was low (π=0.013), and PNC and PESB showed the highest nucleotide diversity among the muriqui populations (π=0.012, Table 1).

Table 1. Genetic diversity indices (*h*: haplotype diversity, *π*: nucleotide diversity, *S:* number of polymorphic sites) based on mtDNA for 11 northern muriqui populations, including locality and forest fragment size, current population size, number of individuals sampled, number of haplotypes and number of unique haplotypes found in this study and in the previous study by Chaves et al. 2011.

Locality (State) [1]	Area size (ha)	Minimum Population	Sampled individuals	h^5	π^5	S^5	N haplotypes (N unique)	N haplotypes (N unique) Chaves et al. 2011
PERD (MG)[2]	35,976	132	23	0.818	0.010	14	8 (2)	8 (5)
PNC (MG-ES) [3]	31,853	N>100 (estimate)	73	0.836	0.012	13	13 (5)	2 (0)
PNI (MG-RJ) [2]	28,084	N<50 (estimate)	1	_	_	_	1 (0)	-
PESB (MG) [2]	13,210	325	15	0.800	0.012	12	5 (1)	7 (5)
REBIO-AR (ES) [2]	3,562.32	N<50 (estimate)	4	_	_	_	2 (1)	-
SMJ (ES) [2]	+2000*	115	42	0.778	0.010	10	6 (4)	7 (5)
PEI (MG) [2]	1,488	2	1	_	_	_	1 (1)	1 (1)
RPPN-FMA (MG)[2, 4]	957	293	52	0.539	0.009	7	3 (3)	3 (3)
RPPN-MS[2,4]	223.74	25	11	0.000	0.000	0	1 (0)	1 (0)
EsF (MG)		1 (isolated female)	1	_	_	_	1 (0)	-
FE (MG) [2]	44	Local extinct	2	_	_	_	1 (0)	1 (0)
Overall			**226**	**0.928**	**0.013**	**23**	**26**	**23**

[1] ES: Espírito Santo; MG: Minas Gerais, RJ: Rio de Janeiro; PERD: Parque estadual do Rio Doce, PNC: Parque Nacional do Caparaó, PNI: Parque Nacional do Itatiaia, PESB: Parque Estadual Serra do Brigadeiro, REBIO-AR: Reserva Biológica Augusto Ruschi, SMJ: Santa Maria do Jetibá, PEI: Parque estatual do Ibitipoca, RPPN-FMA: Reserva Particular do Patrimônio Natural Feliciano Miguel Abdala, RPPN-SM: Reserva Particular do Patrimônio Natural Mata do Sossego, EsF: Esmeralda de Ferros, FE: Fazenda Esmeralda.

[2] Based on Strier et al. 2017,

[3] updated from M. C. Kaizer,

[4] updated from Strier et al. 2019,

[5] calculated only for populations with more than 10 individuals sampled.

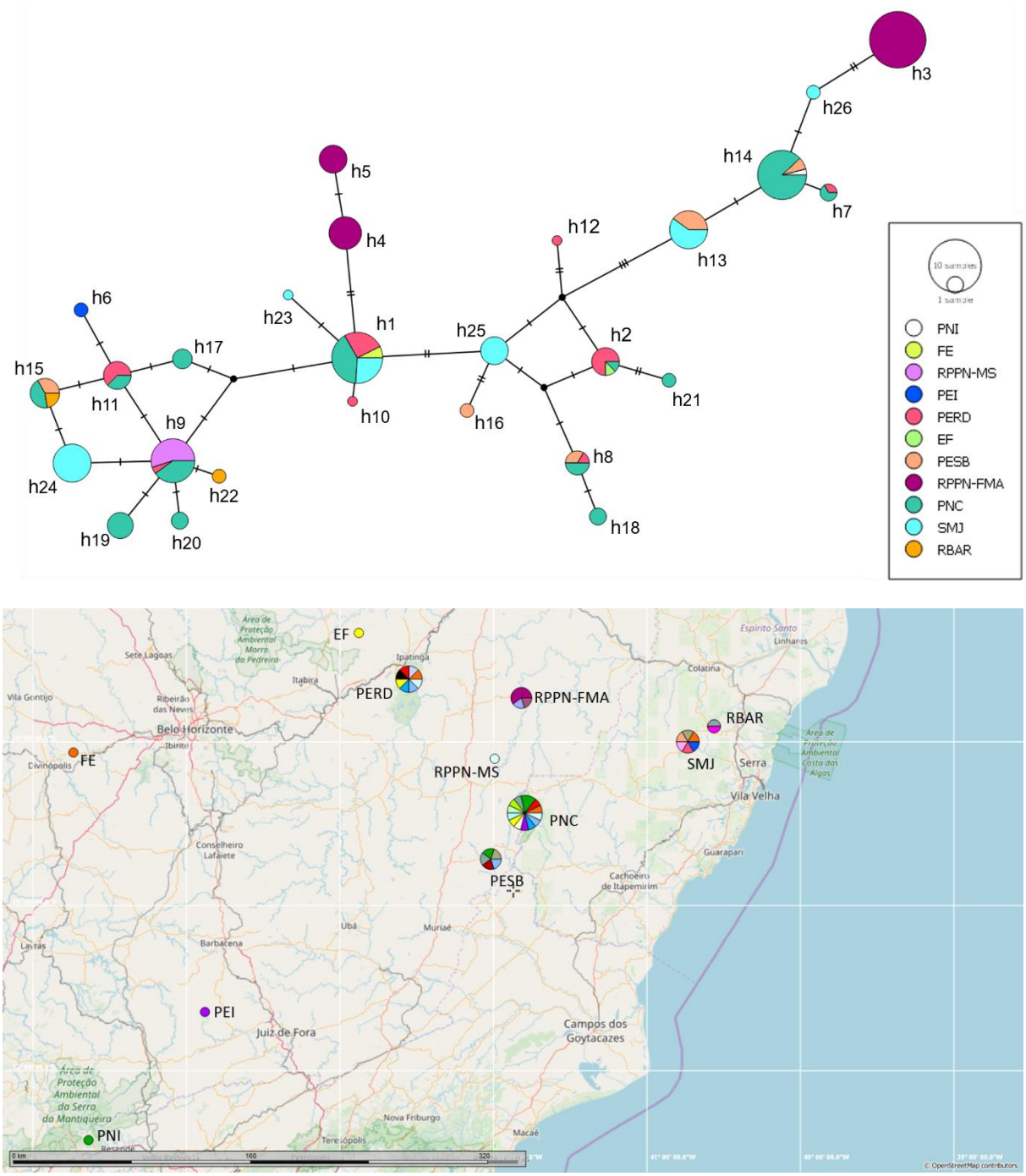

Figure 1. The Median-Joining haplotype network (on the top) and geographic distribution of haplotypes (on the map) based on control region sequences of 11 northern muriqui populations. In the inset, each haplotype is a circle, and circles size represents the relative abundance of each haplotype. Median vectors (small black circles) accounts for a mutation step (transition or transversion), indicated by each hatch-mark on the lines. The geographic distribution and frequencies of haplotypes (pie charts) within muriqui populations can be seen on the map. Colours represent each haplotype, and pie charts size are proportional to the number of samples (n).

The Figure 1 shows the pattern of the haplotype network of muriqui populations, and the geographic distribution of the haplotypes. Overall, from those 26 haplotypes obtained for the 11 populations analysed, 17 of them (65.4%) are unique for only one population: PNC (5), SMJ (4), RPPN-FMA (3), PERD (2) and PEI (1), PESB and RBAR (1). The PNC recovered 13 haplotypes in total, from which 8 haplotypes were shared with at least one distinct population (Figure 1). The haplotype h1, which was found in the currently extinct FE population, was also found in PERD, PNC, and SMJ. Whereas, the haplotype h6 was exclusively found in the PEI population, which has only two extant males. The PNI, which represents the southernmost latitude within the species occurrence range, harbours the haplotype h14, which was only shared with PNC and PESB.

Pairwise F'_{ST} for all muriqui populations with ≥10 individuals sampled showed values ranged from 0.03 (PERD/SMJ) to 0.66 (RPPN-MS/RPPN-FMA; Table 2). Statistically significant differences were found for all pairwise comparisons but PERD/SMJ. In spite of the occurrence of statistically significant differences, low F'_{ST} values (<0.3) recovered in the pairwise comparisons might indicate a weak genetic structuring, whereas the moderate (0.3-0.5) and high values (0.5) suggested the presence of population structure as shown in Table 2. The highest F'_{ST} values were obtained for RPPN-FMA/RPPN-MS (0.66) and PESB/RPPN-MS (0.53). PNC was significantly differentiated from all other muriqui populations analysed but showing moderate F'_{ST} values only when compared to the RPPN-FMA population (0.32).

The genetic divergence due isolation by distance investigated to verify the influence of distances in the populations structuring demonstrated the absence of significant correlation between geographic distances and F'_{ST} values (Mantel statistic: r = -0.526, p-value = 0.90).

Table 2. Pairwise F'_{ST} values for control region of all muriqui population with at least 10 individuals sampled. Colours indicate F'_{ST} values: low (blue), moderate (green), high (red) and non-significant (P>0.05, black).

	RPPN-FMA	PERD	PESB	PNC	RPPN-MS	SMJ
RPPN-FMA	.					
PERD	0.47931	.				
PESB	0.37612	0.14157	.			
PNC	0.31521	0.06968	0.07294	.		
RPPN-MS	0.65967	0.36897	0.53042	0.26547	.	
SMJ	0.43945	0.03212	0.10189	0.04429	0.30154	.

Local-level genetic diversity and structure

Control region (mtDNA)

Within muriqui population at PNC, haplotype h14 showed a high frequency in the population (30%), followed by the h1 (15%). These two haplotypes were found in four of the five muriqui groups sampled (Figure 2). The h7, h19, h20 and h21 were exclusively found only in the VA group, located in the west area of the park. However, this distribution may reflect a sampling bias because this group contributed with 56.2% of the overall samples from PNC.

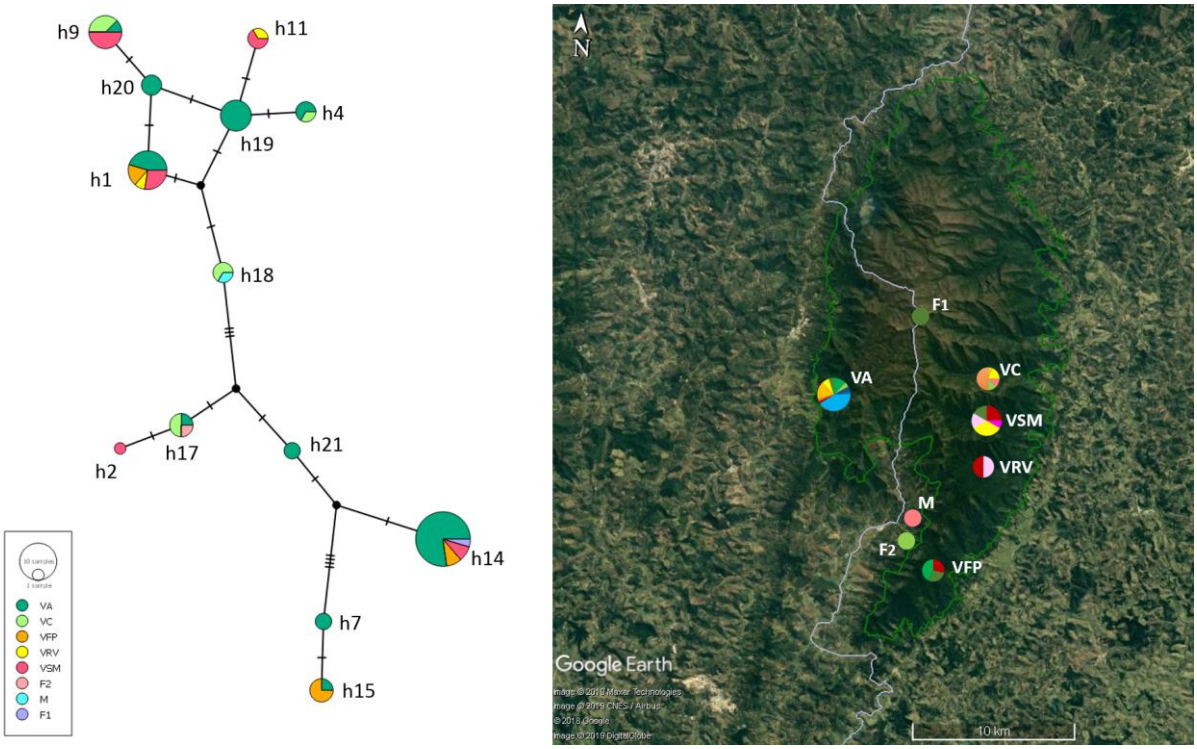

Figure 2. Haplotype network based on control region segment (mtDNA) from northern muriqui population at Caparaó National Park (PNC). In the inset, circles size represents the relative abundance of each haplotype (circle) in the population. Small black circles (median vectors) accounts for a mutation step (transition or transversion), indicated by vertical dashes on the lines. Colours indicate each muriqui social group and isolated individuals (F1, F2, M). On the map, is shown the geographic distribution of shared haplotypes between groups. Each haplotype is represented by a different colour in the pie charts.

The genetic structure of PNC population investigated by the AMOVA yielded a significant but low fixation index between the defined subpopulations east and west (F_{SC} = -0.03, P = 0.008). No genetic structure was found for the five groups (F_{CT} = 0.17, P>0.05) analysed since 85.6% of the genetic variation was found within the groups and only 16.8% of the entire variation recovered was due to differences between the east and west subpopulations in the park (Table 3). The pairwise F'_{ST} analysis considering the five populations (social groups) revealed a significant difference but with low F'_{ST} values between the groups VA and VSM (F'_{ST} = 0.11, P = 0.03) and VA and VC (F'_{ST} = 0.24, P = 0.005; Table 4). No significant correlation was

found between the distances and the F'_{ST} values (Mantel statistic: r = 0.35, p-value = 0.27).

Table 3. AMOVA results based on control region of muriqui population at Caparaó National Park (PNC), Brazil. Bold values indicate statistical significance (p < 0.05).

Analysis	Source of variation	df	Sum of squares	Variance components	Percentage of variation	Fixation indices
Per area (2 groups: east and west)	Among groups	**1**	14.527	0.40445	16.78	**FSC = -0.02859**
	Among populations within groups	3	5.043	-0.057	-2.38	FST = 0.14400
	Within populations	65	134.116	2.06332	85.60	FCT = 0.16779

Table 4. Pairwise F'_{ST} values between muriqui groups at Caparaó National Park (PNC), based on control region (mtDNA). Isolated individuals (n=3) not included. Bold values represent F'_{ST} P-values <0.05.

	VA	**VC**	**VFP**	**VRV**	**VSM**
VA	**.**				
VC	**0.23996**	**.**			
VFP	0.06043	0.03815	**.**		
VRV	0.13808	-0.14937	-0.22599	**.**	
VSM	**0.10735**	0.04844	-0.0512	-0.2409	**.**

Microsatellite markers

The eight microsatellites were successfully amplified for 73 muriqui individuals, revealing a mean number of alleles (Na) of 3.27 for the overall PNC population.

Genetic diversity results are presented in Table 5 for all muriqui groups containing more than seven individuals and for the overall population. For the 8 loci analyzed, the lowest allele richness was found for the VFP group (2.61) and greatest for VA (3.44). The sample-size weighted expected heterozygosity (uH_E) was greatest in VFP (0.66) and lowest in VC (0.53). Overall observed heterozygosity (H_O) was 0.81, with the greatest value found in VFP (0.92) and the lowest in VSM (0.67). Inbreeding coefficients (F_{IS}) ranged from -0.24 for VSM to -0.54 for VFP (Table 5), suggesting significant inbreeding within groups.

Table 5. Genetic diversity estimates for northern muriqui population at Caparaó National Park based on the 8-msat. Number of samples (N), number of alleles (Na), allelic richness (AR), gene diversity (GD), expected (H_E) and observed heterozygosity (H_O), unbiased heterozygosity (uH_E), and inbreeding coefficient (F_{IS}) for muriqui groups with N>7 and for the overall population.

Sampled group	N	Na	AR	GD	H_O	H_E	uH_E	F_{IS}
VA	42	5.00	3.44	0.63	0.81	0.62	0.63	-0.33
VFP	8	3.50	2.61	0.52	0.92	0.61	0.66	-0.54
VRV	2	2.75	-	-	-	-	-	-
I	2	2.13	-	-	-	-	-	-
VSM	12	3.63	3.07	0.55	0.67	0.53	0.55	-0.24
VC	7	2.63	3.33	0.64	0.76	0.50	0.53	-0.53
Overall	**73**	**3.27**	**3.34**	**0.58**	**0.81**	**0.55**	**0.63**	**-0.49**

Population differentiation not assuming Hardy-Weinberg equilibrium (given the results obtained by F_{IS}) revealed low and non-significant differentiation over all groups (F'_{ST} (Theta) = 0.012, 95% CI: -.002–0.027).

Pairwise F'_{ST} indicated differentiation between the group VSM with all other three groups (Table 6): VA (F'_{ST} = 0.019, P = 0.03), VC (F'_{ST} = 0.029, P = 0.03) and VFP (F'_{ST} = 0.045, P = 0.02). However, there was not a significant correlation between F'_{ST} values and geographic distances between groups (r=0.4286, P>0.05). The Bayesian analysis in STRUCTURE identified K=2 as the most likely number of genetic clusters for the PNC population (Table 7). Nonetheless, it seems that there was no

geographic basis to these clusters. A graphical visualization of population stratification through the admixture model is shown in Figure 3.

Table 6. Pairwise F'_{ST} values between muriqui groups at Caparaó National Park (PNC), based on 8-microsat loci for groups with ≥7 individuals. Bold values represent significance F'_{ST} P-values (<0.05).

	VA	VFP	VSM	VC
VA	.			
VFP	-0.0133	.		
VSM	**0.0188**	**0.0288**	.	
VC	0.0171	0.0196	**0.0452**	.

Table 7. The Evanno table output, including the mean log probability LnP(K) and its standard deviation and ΔK (Evanno et al. 2005).

K	Reps	Mean LnP(K)	Stdev LnP(K)	Delta K
1	5	-1223.58	0.238747	—
2	5	-1167.84	1.268069	93.55955
3	5	-1230.74	16.607017	1.646292
4	5	-1266.3	22.430782	2.52956
5	5	-1358.6	64.965491	0.331253
6	5	-1429.38	200.715388	—

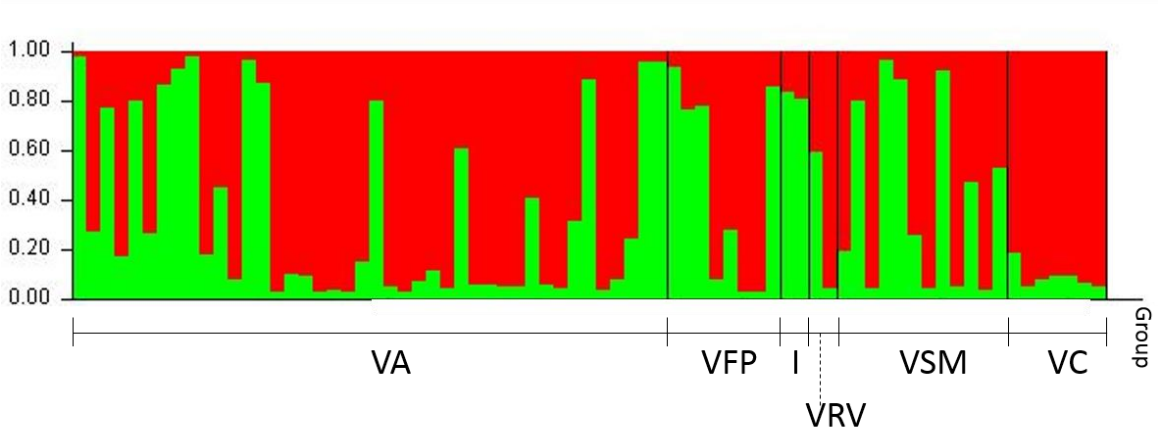

Figure 3. Genetic structure in northern muriqui population at Caparaó National Park (PNC). Bar plots of northern muriqui (n=73) across five social groups (VA, VFP, VRV, VSM, and VC) and isolated individuals (I, pooled together) as inferred by hierarchical runs in STRUCTURE v2.3.4 (Pritchard et al., 2000) based on eight microsatellites loci. Individuals are represented by vertical bars into colours according to their membership coefficients to each cluster (K=2). Vertical black lines separate grouped individuals into populations.

4.4 | DISCUSSION

Despite advances in the field of conservation genetics, which have allowed for successful assessments of wild populations genetic structure using non-invasive samples (e.g. faeces - Hoss et al. 1992, Ericksson et al. 2004, Minhas et al. 2018), the genetic status of the critically endangered northern muriqui remains poorly investigated.

To date, few studies using mtDNA have assessed the genetic diversity of northern muriqui at local (Chaves et al., 2006; Fagundes et al. 2008) and population levels (Chaves et al. 2011), and only one study based on microsatellites assessed paternity skew in only one social group of muriquis (Strier et al. 2011). Here, we used both mtDNA and microsatellite data to assess for the first time the genetic diversity and structure of a high priority muriqui population (PNC) in terms of conservation; including data from a recently discovered group (Kaizer et al. 2016). Combining the control region data obtained for PNC population with additional sequences from Genbank, we also provided an up-to-date assessment on genetic variability across 11 populations of muriquis, covered a large proportion of the species' distribution range.

Overall, northern muriqui genetic diversity based on the mtDNA demonstrated a considerably high haplotype diversity (h=0.928) but nucleotide diversity remained low (π =0.013). This general pattern was found for all populations with more than ten sampled individuals with the exception of the RPPN-MS population (N=11; Fig. 1). A similar picture of genetic diversity was reported for the congeneric species *B. arachnoides* (southern muriqui) based on data recovered for 60 individuals from 10 populations. Analyses using 39 haplotypes (612 bp of the mtDNA control region) yielded a high haplotype (h=0.976) and low nucleotide diversity (π =0.01). The high

haplotype diversity and low nucleotide diversity recovered for these closely related species highlights the importance of taking into account the demographic history of each muriqui species. The low nucleotide diversity has already been associated to bottleneck events followed by population expansion in several species (Yu et al. 2003, Jalil et al. 2008, Allock & Strugnell 2012). For *Brachyteles* spp. this fact might be associated with anthropogenic pressures faced by both endangered species in past centuries, in which northern muriqui populations were drastically reduced and now remain in mostly small, fragmented and less structured forests, whereas the majority of southern muriquis (87.5%) remain in large, undisturbed, and more connected forest (Boubli et al. 2010, Silva Junior et al. 2010).

The Atlantic Forest deforestation has led to the isolation of *B. hypoxanthus* in distinct fragmented areas and 15 extant muriqui populations are currently described, from which here we included data for nine of these populations and added one extinct population. The pairwise F_{ST} comparisons indicated a genetic distinctiveness among all population but non-significant between PERD/SMJ, which represents the widest geographic distance among known muriqui populations. Thus, it is unlikely that the genetic differentiation seen in muriqui populations is a result of isolation by distance as corroborated by the Mantel correlation analyses. Isolated populations might diverge genetically over the generations, regardless of their common origin or connectivity in the past (La Haye et al., 2012).

Despite the importance of the proximity between sites favoring the gene flow, this genetic exchange might not be always associated with short geographical distances and can be influenced by several factors such as a lack of connectivity and presence of physical barriers. The genetic structure can be influenced by different aspects of the species, such as social structure, mating system, sex-biased dispersal,

population size, composition and dynamics (e.g. fission-fusion; Di Fiore, 2003; Milk, 1987). The northern muriqui lives in multi-male and multi-female social groups, characterized by promiscuous mating system and female-biased dispersal pattern (Strier et al. 2006). Likewise, to other primate species with female dispersal, such as chimpanzees and spider monkeys, the expected pattern of the genetic variation is a homogenous distribution among and within populations in the absence of environmental barriers and isolation by distance.

It is potentially this female-biased dispersal pattern (given that mtDNA is inherited maternally) and stable population size in Atlantic Forest refuges during the late Pleistocene climate changes (Carnaval et al. 2009) that best explain the genetic structure observed in northern muriquis based on the mtDNA (Chaves et al. 2011). This is supported by the fact that some haplotypes were shared among geographically distant populations such as the h1 (reference sequence from Genbank AF213966) which was shared among the populations FE (westernmost latitude), PERD, PNC, and SMJ (easternmost latitude). Haplotype sharing among PESB, PNC and PNI (southernmost longitude) also suggest the existence of past gene flow across Atlantic forest before habitat fragmentation. These three populations represent the areas with the highest altitude within the species occurrence range and are inserted in the Mantiqueira Mountain chain, suggesting that there was gene flow along the mountain range in the past.

Regarding the conservation status of the northern muriqui, our study highlights the importance of considering each locality site as a distinct unit for management purposes due to their genetic uniqueness. As an example, here we revealed five newly described haplotypes unique for the PNC population, and one unique haplotype for RBAR. Furthermore, the highest haplotype diversity found in PNC supports the finding

from Chaves et al (2011) that populations occupying larger forest fragments (≥2000 ha, Table 1) harbour the highest haplotype diversity, and the number of unique haplotypes. With ~32,000 ha, the PNC is the third largest area with known populations of muriquis (Strier et al. 2017). The positive relationship between fragment size and haplotype diversity has also been reported in an endemic toad species from the Atlantic Forest (*Rhinella ornate*, Dixo et al. 2009). Regarding the importance of the conservation priority area PNC, this population harboured haplotypes shared with all other muriqui population but RPPN-FMA and PEI, which harbour only unique haplotypes each. It means that some of the haplotypes that were previously considered as unique to some populations were also found in PNC (Chaves et al 2011). This finding stresses the great importance of the PNC population as a potential reservoir of the historical genetic diversity of the species and demonstrates the importance of safeguarding this population and its associated habitat.

In a refined scale, within the PNC population most of the genetic variation resides within social groups when comparing east and west subpopulations as indicated by the AMOVA. However, the pairwise F_{ST} comparisons still indicated a significant genetic distance among the western group VA with the groups VC and VSM, which were the northernmost group sampled in the east area of the park (Fig. 2). Nonetheless, despite the fact that we found a positive correlation, isolation by distance was not a significant factor contributing to genetic structure at the mtDNA level within PNC population. However, our analysis accounted for the shortest straight distance between groups without taking into account the landscape features (e.g. topography, elevation, connectivity, presence of physical barriers). Although the VA group (western area) distances apart ~10km from VC and VSM (both in the eastern area), the Caparaó massif with 2982 m of altitude span longitudinally among them.

Moreover, above 2,000 m of altitude the vegetation type is characterized mainly by altitudinal grasslands while dense rainforest occurs in the lower altitudes (Rizzini, 1979). Thus, the Caparaó massif may have influence on the gene flow within the PNC population by acting as a permeable natural barrier to female dispersal and restricting the connectivity between these social groups. However, additional analyses accounting for the isolation by resistance (IBR) can better elucidate the genetic distinctiveness found between those groups (McRae, 2006). This assumption is also strengthened by the fact that during this study a subadult female muriqui has been found dead at 2982m of altitude, potentially due to a non-successful dispersal attempt. Although it is known that muriquis are able to cross forested patches on the ground (Mourthé et al 2007), they are a forest dwelling primate adapted to suspensory locomotion by brachiation (Hartwig, 2005). Therefore, the absence of forest structure may restrict dispersal over long distances. Forest structure dependence for dispersal over long distances in muriquis was evidenced in the metapopulational of SMJ, where field observations and genetic analysis demonstrated the absence of gene flow among one of the social groups, which was only 6 km apart from the nearest group (Chaves et al. 2011).

Contemporary genetic diversity of PNC population

High levels of genetic diversity within PNC population were obtained for the microsatellite data (Table 5). Similar values for the unbiased heterozygosity (uH_E, ranging from 0.53-0.66) and allelic richness (AR, ranging from 2.61-3.44) were retrieved among social groups. These two measures were reported here since they are corrected by the number of samples within each group. However, some other

primate studies did not include these measures, then for comparative purposes when absent we included comparisons made to H_E values available instead. Overall unbiased heterozygosity (uH_E=0.53) found for the muriquis at PNC, based on the 8 microsatellite panel was similar to or higher than 10 populations of the closely related species *Alouatta caraya* (uH_E, ranging from 0.32 to 0.50; Oklander et al, 2017) which is currently suggested to be identified as threatened due to the population decimation caused by the yellow fever outback. For the endangered lion tamarins (genus *Leonthopithecus*), also endemic to the Atlantic forest and known to have suffered a severe reduction in their natural range, the mean genetic diversity found was H_E = 0.42-0.66 and 0.4-0.7 for the golden lion tamarin (*L. rosalia*; Gravitol et al. 2001) and for four populations of the black faced lion tamarin (*L. crysomelas;* Martins et al. 2011), respectively. However, the H_E values observed in this study were lower in comparison to other engendered primates within the Atelidae family such as spider monkeys (*Ateles belzebuth* – He= 0.68-0.72) and wooly monkeys (*Lagothrix poeppigii* – He= 0.76-0.78; Di Fiore 2009).

Microsatellite results indicated non-significant contemporary differentiation within the PNC population. The social structure of the muriquis – mixed groups, promiscuous mating and low paternity investment (Strier et al. 2011) – probably contribute to a panmictic pattern. However, the F_{ST} pairwise analysis indicated a significant genetic distance among the VSM group with all of the other groups, which was somewhat unexpected. For example, the VSM is located neighboring the VC group and due to the connectivity of the forest and short distance between these localities a high genetic flow is then expected. Thus, to avoid suggesting non-reliable inferences since this pattern might be due the reduced statistical power offered by using a small amount of loci or even by the sample size analyzed. Further analyses

should be conducted adding more markers/samples in order to verify and evaluate the occurrence of this differentiation pattern between the neighbouring groups VSM and VC.

The Bayesian analysis in STRUCTURE indicated the presence of two clusters (Figure 3). However, genetic structure was absent in the PNC population and corroborated with previous analyses which demonstrated the occurrence of gene flow between distinct groups. The most distinct group according to the STRUCTURE plot (VC) was comprised by seven samples from which six were represented by females and could have influenced in the results obtained due to the female dispersal biased.

Given the unexpected contemporary pattern structure found in our study, we highlight the importance of evaluating the role of landscape features into population genetic assessment in PNC (see above when discussing the mtDNA results). In addition, increasing the number of loci and providing addition information about the sample individuals, such as sex-determination (to allow for a more thorough investigation of sex-biased dispersal), will allow for a better understanding of the genetic structure of the PNC population and to identify potential dispersal routes.

Despite being a critically endangered species, the genetic assessment provided here suggests a positive perspective regarding the future conservation of the northern muriquis. The overall genetic diversity remains high and the absence of genetic structure suggests the presence of connectivity and gene flow showing that PNC is potentially a panmictic population. These findings reinforce the importance of keeping large protected areas for species conservation, allowing the maintenance of distinct muriqui social groups and the gene flow between in order to assure the maintenance of genetic diversity. Furthermore, the data here provided can be used

as a baseline for management and conservation purposes such as informing directions for future reintroductions and translocations.

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SUPPLEMENTARY MATERIAL

TS 1. Northern muriqui samples information, including sample ID, type of sample, assigned sex, date of collection and DNA extraction, DNA concentration and respective haplotype inferred by mtDNA markers, and genotypes matches (Duplicate: same individual collected twice during the same day; Recapture: resample of a genetic tagging individual; excluded: not included in the microsatellite analysis).

Sample ID	Group	Sample Type	Sex	Collection Date	Extraction Date	Nucleic Acid (ng/µl)	A260 (Abs)	A280 (Abs)	260/ 280	260/ 230	Hap	Genotype matches
PNC01	I	Tissue	F	Oct-17	Oct-18	29.9	0.598	0.307	1.95	1.61	h14	
PNC02	VA	Fecal		Jan-17	Nov-18	43.4	0.867	0.45	1.93	2.51	h14	
PNC03	VA	Fecal		Jan-17	Nov-18	72.5	1.45	0.765	1.9	2.5	h14	duplicate (PNC03)
PNC04	VA	Fecal		Jan-17	Nov-18	16.4	0.328	0.164	1.99	3.17	h14	
PNC05	VA	Fecal		Jan-17	Nov-18	26.9	0.538	0.265	2.03	4.65	h14	
PNC06	VA	Fecal		Jan-17	Nov-18	14.3	0.286	0.138	2.08	11.84	h14	excluded
PNC07	VA	Fecal		Jan-17	Nov-18	31.7	0.634	0.325	1.95	2.58	h14	
PNC08	VA	Fecal		Jan-17	Nov-18	25	0.501	0.262	1.91	5.6	h19	
PNC09	VA	Fecal		Jan-17	Nov-18	20.4	0.408	0.194	2.1	13.16	h19	
PNC10	VA	Fecal		Jan-17	Nov-18	27	0.54	0.297	1.82	1.93	h14	
PNC11	VA	Fecal		Fev-17	Nov-18	13.8	0.275	0.146	1.89	3.75	h19	
PNC12	VA	Fecal		Feb-17	Nov-18	32.6	0.651	0.339	1.92	2.5	h19	
PNC13	VA	Fecal		Feb-17	Nov-18	5.4	0.108	0.067	1.61	3.34	h1	
PNC14	VA	Fecal		Feb-17	Nov-18	15.9	0.317	0.166	1.91	6.59	h8	
PNC17	VSM	Fecal		Fev-17	Nov-18	2.5	0.05	0.027	1.87	-5.02	h9	excluded
PNC18	VSM	Fecal		Fev-17	Nov-18	4.3	0.086	0.052	1.65	0.95	ns	
PNC19	VRV	Fecal		Fev-17	Nov-18	5.1	0.102	0.05	2.03	15.21	h11	
PNC20	VRV	Fecal		Fev-17	Nov-18	2	0.039	0.016	2.44	6.71	h1	
PNC21	VSM	Fecal		Fev-17	Nov-18	13.9	0.277	0.133	2.08	0.8	h1	
PNC22	VSM	Fecal		Fev-17	Nov-18	6.8	0.136	0.07	1.94	-14.78	h9	
PNC24	VSM	Fecal		Fev-17	Feb-19	76.9	1.539	0.787	1.95	1.94	h1	
PNC25	VSM	Fecal		Fev-17	Nov-18	4.9	0.098	0.048	2.05	2.46	h1	
PNC28	VA	Fecal		Mar-17	Nov-18	3.5	0.069	0.041	1.67	6.3	h1	
PNC29	VSM	Fecal		Mar-17	Nov-18	8.6	0.172	0.092	1.87	2.93	h11	
PNC30	VSM	Fecal		Mar-17	Nov-18	8.1	0.162	0.08	2.03	1	h14	
PNC31	VSM	Fecal		Mar-17	Nov-18	8.6	0.172	0.092	1.87	3.33	h9	
PNC32	VSM	Fecal		Mar-17	Nov-18	-0.5	-0.011	-0.006	1.95	0.26	h11	
PNC33	VSM	Fecal		Mar-17	Nov-18	3.4	0.067	0.024	2.79	-2.53	h9	
PNC34	VSM	Fecal		Mar-17	Nov-18	11.6	0.233	0.122	1.9	2.14	h9	recapture (PNC31)
PNC35	VSM	Fecal		Apr-17	Nov-18	3.1	0.063	0.031	2	2.22	h14	
PNC38	VA	Fecal		May-17	Nov-18	3	0.061	0.035	1.72	-3.49	h1	
PNC39	VA	Fecal		May-17	Nov-18	11.5	0.231	0.119	1.94	1.05	h19	
PNC41	VSM	Fecal		Jun-17	Nov-18	4.8	0.096	0.062	1.56	0.83	h2	
PNC46	VFP	Fecal		Jun-16	Nov-18	25	0.5	0.261	1.92	2.3	h14	
PNC47	VFP	Fecal		Jun-16	Nov-18	58.4	1.168	0.608	1.92	1.95	h14	

Sample ID	Group	Sample Type	Sex	Collection Date	Extraction Date	Nucleic Acid (ng/µl)	A260 (Abs)	A280 (Abs)	260/ 280	260/ 230	Hap	Genotype matches
PNC48	VC	Fecal		Apr-16	Feb-19	9.3	0.185	0.058	3.21	1.35	h9	
PNC49	VC	Fecal		Apr-16	Feb-19	11.5	0.23	0.086	2.69	1.1	h18	
PNC50	VC	Fecal		Apr-16	Feb-19	1.9	0.038	-0.007	-5.2	0.72	h9	
PNC51	VC	Fecal		Apr-16	Feb-19	11.2	0.225	0.088	2.55	0.65	h9	
PNC52	VC	Fecal		Apr-16	Feb-19	8.9	0.177	0.07	2.54	1	h17	
PNC53	VC	Fecal		Apr-16	Feb-19	1.4	0.028	-0.009	-3.22	0.19	h17	
PNC54	VC	Fecal		Apr-16	Feb-19	-1.5	-0.03	-0.043	0.7	1.07	h18	
PNC75	VA	Fecal		Sep-17	Nov-18	-0.1	-0.001	0.005	-0.24	0.02	h17	
PNC76	VA	Fecal		Sep-17	Nov-18	4.7	0.095	0.055	1.74	0.33	h14	
PNC78	VA	Fecal		Fev-18	Nov-18	19.9	0.398	0.217	1.84	1.71	h9	
PNC79	VA	Fecal		Fev-18	Nov-18	71.6	1.431	0.716	2	1.63	h14	
PNC80	VA	Fecal		Fev-18	Nov-18	27.1	0.541	0.329	1.64	1.85	h1	recapture (PNC28)
PNC81	VA	Fecal		Fev-18	Nov-18	29.9	0.597	0.314	1.9	2.02	h1	excluded
PNC82	VA	Fecal		Fev-18	Nov-18	30.5	0.609	0.307	1.98	1.82	h14	
PNC83	VA	Fecal		Fev-18	Nov-18	175.5	3.511	1.8	1.95	1.93	h14	
PNC84	VA	Fecal		Fev-18	Nov-18	49.3	0.985	0.55	1.79	1.8	h1	
PNC85	VA	Fecal		Fev-18	Nov-18	69.9	1.397	0.703	1.99	2.04	h14	
PNC86	VA	Fecal		Fev-18	Nov-18	33.5	0.67	0.363	1.85	2	h14	
PNC87	VA	Fecal		Fev-18	Nov-18	16.3	0.326	0.183	1.78	1.9	h1	
PNC88	VA	Fecal		Fev-18	Nov-18	151.1	3.022	1.521	1.99	2.11	h14	
PNC89	VA	Fecal		Fev-18	Nov-18	46.7	0.935	0.488	1.91	1.97	h14	
PNC90	VA	Fecal		Fev-18	Nov-18	76.5	1.529	0.766	2	2.12	h14	
PNC91	VA	Fecal		Fev-18	Nov-18	191.7	3.834	1.94	1.98	2.32	h14	
PNC92	VA	Fecal		Fev-18	Nov-18	269.4	5.388	2.785	1.93	2.14	h20	
PNC93	VA	Fecal		Fev-18	Nov-18	42.4	0.848	0.42	2.02	1.51	h14	
PNC95	VFP	Fecal		Mar-18	Nov-18	22.9	0.458	0.234	1.95	2	h15	
PNC96	VFP	Fecal		Mar-18	Nov-18	2.7	0.054	0.032	1.69	-2.32	h15	
PNC97	VFP	Fecal		Mar-18	Nov-18	3.3	0.066	0.032	2.07	-1.17	ns	
PNC98	VFP	Fecal		Mar-18	Nov-18	0.2	0.004	0.003	1.35	-0.04	h1	
PNC99	VFP	Fecal		Mar-18	Nov-18	10.5	0.21	0.104	2.02	5.59	h15	
PNC100	VFP	Fecal		Mar-18	Nov-18	18.1	0.362	0.183	1.97	2.85	h1	
PNC102	VA	Fecal		Mar-18	Nov-18	17.1	0.342	0.192	1.78	1.99	h14	
PNC103	VA	Fecal		Mar-18	Nov-18	38.9	0.778	0.391	1.99	1.44	h19	
PNC104	VA	Fecal		Mar-18	Nov-18	11.9	0.237	0.139	1.71	0.49	h15	
PNC106	VA	Fecal		Mar-18	Nov-18	48.7	0.974	0.499	1.95	2.35	h19	
PNC107	VA	Fecal		Mar-18	Nov-18	55.5	1.111	0.577	1.92	1.99	h20	
PNC108	VA	Fecal		Mar-18	Nov-18	42.4	0.849	0.429	1.98	2.53	h20	
PNC109	VA	Fecal		Mar-18	Nov-18	33.7	0.674	0.344	1.96	2.18	h21	
PNC110	VA	Fecal		Mar-18	Nov-18	76.2	1.525	0.799	1.91	2.23	h21	
PNC111	VA	Fecal		Mar-18	Nov-18	15.9	0.318	0.159	2	1.99	h7	
PNC112	VA	Fecal		Mar-18	Feb-19	4	0.08	0.003	25.19	0.34	ns	
PNC113	VA	Fecal		May-18	Feb-19	2.1	0.043	0.004	9.58	1.41	h7	
PNC114	VA	Fecal		May-18	Feb-19	16.9	0.337	0.146	2.3	1.97	h8	
PNC115	I	Fecal	F	Ago-18	Feb-19	10.4	0.208	0.094	2.21	0.64	h17	

Sample ID	Group	Sample Type	Sex	Collection Date	Extraction Date	Nucleic Acid (ng/µl)	A260 (Abs)	A280 (Abs)	260/ 280	260/ 230	Hap	Genotype matches
PNC116	I	Tissue	M	Oct-18	Jun-19	79	1.581	0.824	1.92	2.14	h18	

TS 2. Additional sequences from GenBank used in this study including muriqui population and number of individuals represented.

Population	N individuals	GenBank Accession Number	Reference
PERD (MG)	23	JF769865.1-JF769866.1	Chaves et al. (2011)
PNC (MG-ES)	2	JF769865.1-JF769867.1	Chaves et al. (2011)
PNI (MG-RJ)	1	MG365984.1	Chaves (2017)
PESB (MG)	15		Chaves et al. (2011)
REBIO-AR (ES)	4	MG366066.1 - MG366069.1	Chaves (2017)
SMJ (ES)	42	MG366070.1 - MG366110.1	Chaves (2017)
PEI (MG)	2	MG365986.1 - MG365987.1	Chaves (2017)
RPPN-FMA (MG)	52	MG365988.1 - MG366065.1	Chaves (2017)
RPPN-MS	11	JF769867.1	Chaves et al. (2011)
EsF (MG)	1	MG365985.1	Chaves (2017)
FE (MG)	2	JF769866.1	Chaves et al. (2011)

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TS 3. Primers set and description of microsatellites loci studied for northern muriqui (*Brachyteles hypoxanthus*) population at Caparaó National Park, Brazil, including repeat motif, primer sequence, fluorescent dye, number of PCR replicate, size-range in this study and in the literature, species origin and primers reference.

Locus	Repeat Motif	Primers (5'→3')	Dye	Replicate	Size-range this study	Size-range*	Species origin	Reference
Multiplex I								
LL113	$(GT)_{15}$	GCAAAACTCCCCTGTGACTG CCCACTCTCCTCCACAAAGG	FAM	3	167-189	181–186	*Lagothrix lagotricha*	1
SB30	$(CA)_9$-AT-$(CA)_{11}$	TAAAGTTAAGATTGGATTTCAC GCAGAAAAACCTAACAATACA	FAM	4	83-103	83-103	*Saguinus bicolor*	2
D5S111	CA	GGCATCATTTTAGAAGGAAAT ACATTTGTTCAGGACCAAAG	HEX	3	161-173	162-174	*Homo sapiens*	3
Multiplex II								
LL1110	$(GT)_2$	GGTGAATGAGAGAATCAAAG TATGTTCCACAGTAGAAAGC	HEX	3	212-219	218-222	*Lagothrix lagotricha*	1
SB38	$(CA)_{19}$	GCCTCAATGGGTTTTAACC AGAACGAGTCTGTATCTTGA	FAM	3	126-142	126-144	*Saguinus bicolor*	2
D17S804		GCCTGTGCTGCTGATAACC CACTGTGATGAGATGTCATTCC	HEX	4	151-159	152-162	*Homo sapiens*	4
Multiplex III								
LL1115	$(GT)_3(GA)_1(GT)_5$	GCTCATATTCATACATCCCTTGG TTTGCTTGCTCATTCATTGC	HEX	3	194-206	196–208	*Lagothrix lagotricha*	1
LL1118	$(CA)_2(TA)_1(CA)_{17}$	TTTCTCCCTCTCAGATTACCAG CCTTGAGGTTTTTGGGTTCC	FAM	3	130-156	142–166	*Lagothrix lagotricha*	1

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2. Bohle UR, Zischler H (2002) Polymorphic microsatellite loci for the mustached tamarin (Saguinus mystax) and their cross-species amplification in other New World monkeys. Mol Ecol Notes 2:1e3.

3. Weber JL, Kwitek AE, May PE (1990) Dinucleotide repeat polymorphisms at the D5S107, D5S108, D5S111, D5S117 and D5S118 loci. Nucleic Acids Res 18:4035.

4. Weissenbach J, et al. (1992) A second-generation linkage map of the human genome. Nature 359:794e801.

TS 4. Absolute and relative frequencies of haplotypes per each northern muriqui population (and social group, when known).

Haplotype	RJ	MG										ES								Overall
	PNI	FE	EF	PEI	PERD	PESB	RPPN-FMA				RPPN-MS	PNC						SMJ	RBAR	
				ML	LP	MTP	M	J	M2	N		VA	VC	VFP	VRV	VSM	I*			
h1		2			7							5		2	1	3		7		27
h2			1		6											1				8
h3							33													33
h4							10		1											11
h5							8													8
h6				2																2
h7					1							2								3
h8					1	2						2	1							6
h9					1						11	1	3			4				20
h10					1															1
h11					5										1	2				8
h12					1															1
h13						6												9		15
h14	1					2						17		2		2	1			25
h15						3						1		3					2	9
h16						2														2
h17												1	2				1			4
h18													2				1			3
h19												7								7
h20												3								3
h21												2								2
h22																			2	2
h23																		1		1
h24																		15		15
h25																		8		8
h26																		2		2
N° individuals	1	2	1	2	23	15	51		1		11	42	8	7	2	12	3	42	4	226
N° haplotype	1	1	1	1	8	5		3			1				13			6	2	26
N unique haplotype	0	0	0	1	2	3		3			0				5			4	1	20
N_{hap}/N_{ind} (population)	1	0.5	1	0.5	0.35	0.41		0.06			0.09				0.19			0.14	0.50	0.12

Brazilian States: RJ: Rio de Janeiro, **MG**: Minas Gerais, **ES**: Espírito Santo
Population name: PNI: Parque Nacional do Itatiaia, FE: Fazenda Esmeralda (currently extinct), **EsF**: Esmeral de Ferros, **PEI**: Parque Estadual do Ibitipoca, **PERD:** Parque Estadual do Rio Doce, **PESB**: Parque Estadual da Serra do Brigadeiro, **RPPN-FMA:** Reserva Particular do Patrimônio Natural Feliciano Miguel Abdala, **RPPN-MS**: Reserva Particular do Patrimônio Natural Mata do Sossego, **PNC:** Parque Nacional do Caparaó**, SMJ:** Santa Maria de Jetibá, **RBAR**: Reserva Biológica Augusto Ruschi
Group name: ML: Mata do Luna; **LP:** Lagoa Preta; **MTP:** Matipó, **M:** Matão, **J:** Jaó, **M2:** Matão 2, **N:** Nadir; **VA**: Vale do Aleixo, **VC:** Vale do Calçado**, VFP**: Vale do Facão de Pedra, **VRV**: Vale do Rio Veado, **VSM:** Vale Santa Marta, **I***: isolated individuals (male: 1, female: 2).

Chapter V

GENERAL DISCUSSION

Chapter V

General Discussion

5.1 | Thesis Summary – Main Findings

Our understanding of primate populations has increased markedly over the last 40 years. Yet surprisingly, the Neotropical region, which holds the world's largest primate biodiversity, is also the least studied when compared to other regions (Estrada et al., 2017). As Neotropical primates are imperiled by the expanding anthropogenic pressures effective tools to collect baseline information and monitor primates populations is increasingly need (Estrada et al. 2018; 2019; Garber et al. 2009). Noninvasive monitoring tools for conservation, including camera traps (Gregory et al. 2017; Rivas-Romero et al. 2015), passive acoustic devices (Duarte et al., 2018), drones with thermal imaging (Spaan et al., 2019), and molecular biology (Lam et al. 2019; Ruiz-Garcia et al. 2019; Moreira et al. 2010; Di Fiore 2003) are becoming more widespread, leading to a significant advancement in the identification, detection, monitoring and understanding of Neotropical primates diversity and populations.

This thesis demonstrates the application of noninvasive methodologies to overcome the gap in knowledge on the population of a critically endangered Neotropical primate – the northern muriqui (*Brachyteles hypoxanthus*). By demonstrating the effectiveness and application of two noninvasive biomonitoring techniques, I contributed to the advancement in understanding regarding the application of these methods to arboreal primate studies and more specifically to the northern muriqui conservation. I have provided empirical evidence of a high priority

site for species conservation, previously neglected due to its difficult-to-access forest, by assessing the group size and demographic composition of two unhabituated resident muriqui groups and evidencing knowledge of its genetic diversity and variation at a species and population level.

As discussed throughout this thesis, northern muriqui was historically distributed throughout the Atlantic Forest of Brazil (Aguirre 1971), but deforestation and anthropogenic pressures (e.g. illegal hunting) lead this species to isolation in small to medium size fragments (Melo et al. 2018). Thus, population assessment of remnant populations and questions regarding the effects of forest fragmentation on the genetic status of the muriquis populations are now recognized in the National Action Plan for Muriquis Conservation (PAN-Muriqui; Jerusalinsky et al. 2011; Strier et al. 2017).

Because of the urgency of up-to-date data about remnant primates populations and the limited resources that most of conservation projects face, select an effective approach is a key point when define a survey (Campbell et al. 2016). Our findings demonstrated that arboreal camera trapping can be considered as a more effective tool in detecting and monitoring a range of arboreal primates including different species with varied body sizes and home ranges when compared to the most traditional ground-based technique, - transect line (Buckland et al., 2010). Additionally, we show that this novel method brings the advantage of saving a significant amount of human labour and money. Thus, these limited resources, which are crucial for scientific research and wildlife conservation, can be then be redirected towards other important purposes (Schipper, & Rovero, 2017; Stem et al. 2005).

While a range of empirical evidences and directions on camera trapping design are available to terrestrial camera trapping (Kolowski & Forrester 2017;Rovero et al. 2014; Hamell et al. 2013; Silveira et al. 2003; Swann et al. 2011; O'Connell et al. 2011),

few is known about influence of camera-trapping on detection probability of arboreal species. By highlighting the influence of different components of the arboreal camera trapping design (i.e. number, spacing and positioning in the three dimensional environmental of canopy), we made a key step to start to fill this gap. We provided a key step expect that these evidences can be used as a baseline for future studies, providing information for study plan and design. Further, filling the gaps on the surveying of unhabituated muriquis populations and contributing to scaling up (spatially and temporally) similar studies in tropical forests canopy.

Collecting demographic data on animal populations are essential to define the conservation status of a population/species and also allow to better design and evaluate conservation strategies through time (IUCN 2017; Rovero et al. 2015). Then a closely systematic monitoring of primate populations is usually adopt in primatology (Strier et al. 2017; Setchell & Curtis, 2011; Strier & Mendes, 2009). However, despite its great importance, the implementation of this closely systematic monitoring (e.g. habituation) is questioned due to its ethical issues (e.g. disease transmission, hunting pressures) and also, affected by a range of factors (i.e. permission, feasibility, logistic, accessibility, personal and financial resources; Strier et al. 2017), which may prevent its implementation. To overcome these issues, I have used an integrated approach of camera trapping and genetic tagging, which have been proven to be a powerful and effective biomonitoring tool to population assessment in a range of taxa (Whittington & Sawaya. 2015; Rachmat et al. 2011; Anille & Devillard 2008), despite the fact that they are rare for arboreal primates (Arandjelovic & Vigilant 2018; Gregory et al. 2014, 2017). By counting individuals in temporal events based on camera data or individually identification in genetic tagging we overcome the risks of over-estimate population size. Our multidisciplinary approach is widely applicable to other priority northern

muriqui populations and also to its congener species, the southern muriqui (*B. arachnoides*), which do not present facial depigmentation and are more difficult to be counted by traditional ground techniques.

Despite the conservative estimate obtained by genetic tagging, our results supported the reliability of our integrative approach (Rachmat et al. 2011) to study unhabituated muriquis, and raised important information that will be used to drive new research and conservation actions in this protected area. For example, our results showed a substantial difference in the minimum group size between the two areas (VA: 350 ha, 42 individuals; VSM: 1400 ha, 23 individuals), which will drive new investigations, especially regarding the role of this protected area on northern muriqui conservation (Galetti et al. 2015; Rodrigues et al. 2004; Parrish et al. 2003).

In this study we improved the knowledge on the distribution of northern muriquis in the area since the species occurrence was reconfirmed for all the sites reported in the literature (Chapter 1, Box 1), and muriquis were also found occurring in two new sites within the park: VRN - *Vale do Rio Norte*, and VRP - *Vale do Rio Preto*. In summary, the northern muriqui population at PNC is mainly distributed on the eastern side of the park (VRN, VC, VSM, VRV, VFP, VRP), while a single group is known to occur in the western area (VA). In face of the isolation of unique group in the west side, genetic analyses were crucial for understanding the connectivity of these two main areas and to provide further information for management purposes.

Conservation and management of species are known to be more effective when underpinned by the application of genetic tools (Lamb et al. 2019; Frankham 2010; 1995; Allendorf et al. 2007). However, despite the great advances in molecular ecology and the increasingly feasibility and cost effectiveness of these noninvasive approach its application towards the conservation of northern muriqui is still lacking

(Strier et al. 2017; Chaves et al. 2011). Here, we provided new insights on the historical genetic diversity of northern muriqui populations, by contributing with new 73 individuals from the PNC population, which was poorly investigated (Chaves et al. 2011). We revealed that PNC population preserves the highest haplotype diversity among northern muriqui populations and more importantly, this population harbors five unique haplotypes identified in this study. Genetic uniqueness is one of the criteria to prioritize the demographic monitoring for muriquis populations (Strier et al., 2017; Chaves et al., 2011), thus, because the quantity and differentiation of the haplotypes found exclusively in the PNC population it might be prioritized as discrete management population (Moritz 1994).

Furthermore, despite the existence of a putative geographical barrier (the Caparaó massif mountain) between two regions of the park, our findings suggested that PNC muriqui population have potential to be considered a genetic panmictic population (Wright 1949). It seems there is clearly gene flow to prevent genetic variation, and therefore muriquis social groups are not genetically isolated. However, despite the lack of isolation by distance in PNC population, others landscape features should be investigate in future studies (e.g. elevation; Reis et al. 2018; Manel et al. 2003). Our results contrasts with expectations for genetic diversity recovered from small populations (Frankham 1996). Genetic diversity across different species tend to be reduced in those populations occupying small areas (Zuidema, 1996).

However, despite the high genetic diversity revealed for the 11 northern muriqui populations analysed in this study, there is no gene flow among them, and habitat connectivity should be recognized as priority action to this species conservation (Fagundes et al. 2005). In addition, high genetic diversity seen in muriqui populations do not reflect the rapid pace of anthropogenic pressures, probably because habitat

fragmentation and population shrinkage occur at a faster pace compared to the slow life history traits of muriquis (Pope 1998; Strier 1992; Fagundes et al. 1998; Chaves et al. 2011). This also highlights the importance to study the genetic diversity in both local and global scales and when possible, including comparisons between generations.

5.2 | *Challenges and Limitations*

For many years, the northern muriqui population at Caparaó National Park remained neglected especially due the remoteness of the forest and difficult-to-access terrain. One of the main advantages to apply noninvasive monitoring techniques, such as camera trapping and genetic censusing, is to monitor areas where the traditional techniques are not feasible to be implemented (Bowler et al. 2018; Whitworth et al. 2016; McCarthy, et al. 2015; Steyer et al. 2016; Borthakur et al. 2016). However, even applying these noninvasive techniques in our study pose significant challenges, and a range of questions are still unanswered regarding a population assessment of northern muriqui in this priority area.

The Caparaó National Park is one of the largest federal protected area in the Atlantic Forest. With ~32,800 ha, the park is located across two Brazilian states, reaching out ten distinct districts (ICMBIO 2015). The main challenge of PNC managers rely on surveillance of this large area with few personal and financial investment from the Brazilian government along an increasingly anthropogenic pressures (i.e. poaching, palm harvesting, selective logging, forest fires, unsustainable tourism, and an illegal non-native indigenous settlement). Similarly to other protected areas in Atlantic Forest (Galetti et al., 2017), PNC is not surveillance frequently and

uncontrolled illegal hunting poses both threats to wildlife as risks to fieldwork researchers. Therefore, a replicated system of transects or a grid was prevented from being applied due to the risks to increasing human pressures inside the area.

While the adoption of noninvasive biomonitoring techniques such as camera trapping and genetic tagging can overcome the limitations of traditional techniques on arboreal primate population assessment, a limited progress can be achieved if sampling designs are not adequate (Hebblewhite & Haydon 2010). Temporal and spatial sampling design can affect the number of samples collected and the possibility to perform further statistical analysis and to capture-recapture models (Woodruff et al. 2015). Therefore, despite the advantages of opportunistic genetic sampling (e.g. reduce cost, Arandjelovic et al. 2010), and the arboreal camera trapping (Gregory et al. 2014), our study design reduced the power of our analysis. Further, while camera have the advantage of being placed for extended time period within an area, the genetic-based monitoring would require more extensive and systematic sampling than was undertaken in this study.

Additional challenges are regarding studying genetics of this species rely on obtaining of noninvasive samples due to difficulties in accessing remote areas, the need of optimizing protocols for the preservation of samples when freezing is not a feasible option (e.g. evaluating the inclusion of buffers such as RNA latter), and the costs associated with molecular analyses. Furthermore, despite the great contribution of the National Action Plan for Muriquis Conservation through the establishment of protocols and recognition of the need for additional genetic studies, the lack of a public sample collection (e.g. tissue, faeces) hampers advancements in the knowledge regarding these species since most of the samples obtained remain restricted to few institutions. The data obtained from the NCBI (Genbank) public databases contributes

by providing additional DNA sequences as demonstrated in this study for the mtDNA. However, when not including a more complete information (i.e. locality sampled, species analyzed, region sequenced) the use of this data might be hindered, and for some specific markers (e.g. microsatellites as used here) calibration is required across different laboratories (Haaland et al. 2011; Broquet & Petiti, 2004).

Furthermore, due the opportunistic genetic sampling the number of samples were not homogenous among social groups (i.e. some groups have been represented with more than 10 faecal samples while other groups with less samples), thus the comparison between groups might have been influenced by the number of analyzed samples. A more reliable and robust data could be obtained if a higher number of samples were analyzed for the studied groups and also, containing a more comparable data across the localities studied. One option to overcome this limitation could be achieved by including a higher number of loci analyzed. Despite having 17 described microsatellites loci for the muriqui, due to the time required for optimizing the laboratory protocols and reducing the still high financial costs of amplification and genotyping this studied included only eight out of the 17 described. Due the high variability of microsatellites, we were able to identify and differentiate muriqui individuals using 8-loci with statistical confidence (PI*sibs* and PI*ave*; Waits et al. 1992), allowing for the first time the quantification of minimum population size from two resident groups of muriquis using noninvasive samples. However, additional data will be included for the remaining 9 loci in order to strength the data provided in this study.

An additional constraint for studies being currently conducted in Brazil is the bureaucratic protocols needed to be followed prior sample collection and analyses. A recent law established a requirement of an extensive work frame to be followed and despite the importance of this to safeguard the Brazilian biodiversity data, the lack of

association between the organizations responsible for the permits delay and hamper the development of scientific studies in this country (Alves et al. 2018; Bockmann et al. 2018; Silva & Oliveira 2018;). As an example, for the present study the collection (obtained from the SISBIO) and import licenses (DEFRA) were obtained in 2016, but the recently created SISGEN was still in implementation phase and was only made available in 2018 when all the samples could be exported to the UK for further molecular analyses.

5.3 | *Future Directions*

Since arboreal camera trapping is in its infancy and the understanding of this method in the three-dimensional environment of forest canopy is far from our understanding in the two-dimensional environment of ground, a range of opportunities to evaluate the applications of canopy camera traps are available and should be tested. A brighter future for arboreal camera trapping as a noninvasive biomonitoring tool for arboreal primate population assessment will have to be implemented if survey design is carefully addressed and standardized across sampling seasons and sites, relevant trends in population can be detected over time.

Camera trap data management is crucial to save limited resources (i.e. time and money, Glover-Kapfer et al. 2019) and to implement an effective arboreal camera trap biomonitoring programmes. Recent advances of machine learning (i.e. automated identification) may improve image classification and reduce time subsequent analyses (Norouzzadeh et al. 2017; Tabak et al. 2019; Loos & Pitzer et al.2012). Furthermore, since muriquis can be uniquely identified in camera trap data, due to their distinct patterns of facial depigmentation; they can be used as a model species in capture-

recapture models in arboreal camera trapping. In this case, preference might be given for camera traps models that generate high-quality dataset, thus improving images classification and individual recognition.

Although cleaning the surrounding of camera traps in the canopy forest help to minimize interference of branches and leaves (Gregory et al. 2014), arboreal camera trapping still generate a large amount of blank images as supported by our study. However, future work may take advantage of this large amount of data to track habitat changes and the complex ecological interactions in the forest canopy. Acoustic data can be extracted from video, allowing to a range of studies from species identification to soundscape ecology (Towsey et al. 2014; Pijanowski et al. 2011). But an additional use from these data can be based on the fact that camera traps provide real time data including date, time and temperature, allowing to track changes in climate and forest phenology (Morisette et al. 2008), and assess the effects of climate change (i.e. elk, Cervus Canadensis; Brondie et al. 2014). Ongoing climatic changes is a silent threat that may exacerbate the pressures on primate populations and the resilience of tropical forests and protected areas (Lima et al. 2019; Ribeiro et al. 2016). Since climate changes (e.g. increasing temperature and drought) effects local vegetation and thus, will might alter primates diversity both spatially and temporally (Carvalho et al. 2019; Lima et al. 2019), long-term use of arboreal camera trapping may provide important insights on the effects of climate change on northern muriqui population. Further, due the geographic importance of PNC, which is the highest altitudinal range of the species (Strier et al. 2017), a comparative evaluation with other northern muriqui populations would be more valuable and possible with the adoption of standardized protocol (pre-designated intervals).

A better contribution by both arboreal camera trapping and genetic tagging to

capture–recapture method and population assessment of unhabituated muriqui populations could be performed if validating these techniques in an area with known number of muriquis. Based on this validation, improvements in study design, intensity of monitoring and standardized protocol could be defined, allowing their applicability for all northern muriqui populations, despite ecological features of each site (Arandjelovic et al. 2010).

As highlighted in this study, local populations may play a significant role in the global conservation of this species. Despite the inclusion of mtDNA data for 11 out of the 15 known populations, the information provided by this genetic marker is very limited and thus, a better assessment should include additional data obtained by applying a different range of methods/markers (e.g. nuclear DNA data provided by microsatellites) for all the remaining populations (Whiten et al. 2008; Selkoe & Toonen 2006). Although mtDNA data is important for investigating broad scale patterns of genetic diversity and differentiation (Jensen-Seaman & Kidd 2001), there is a lack of information at genetic markers which give information about more contemporary events such as anthropogenic-caused fragmentation. The use of genetic markers such as microsatellites or single nucleotide polymorphisms (SNPs) give a better picture of contemporary metapopulation dynamics in species with fragmented ranges (e.g. Weckworth et al. 2012; Carroll et al. 2018). Additionally, a higher number of analyzed loci (e.g. amplification of the 17 loci used by Strier et al., 2011) when compared to the present study (eight loci) is also required in order to provide a more reliable and robust data.

Furthermore, understanding the genetic diversity fluctuations in the muriqui populations might contribute to investigate the influence of several factors in this species responses and survival. Then, additionally, the recovery from historical data

from museum specimens by using ancient DNA (Higuchi et al. 1984) may improve our understanding of consequences of small and isolated population sizes of this critically endangered species. Dozens of muriqui specimens have been collected during naturalists' expeditions in the last century and are now spread across European and North American museums (Aguirre 1971). Thus, by sequencing the control region of the museum specimens collected up to more than 100 years ago (e.g. 1829, AMHN) and comparing to the currently population we will be able to quantify the genetic diversity changes and verify its association to the population decline. Recent studies comparing DNA from historical samples with modern DNA samples documented the effects of population decline in the reduction of genetic variation over time in other critically endangered species (*condor*: D'Elia et al. 2016; *gorillas*: Van der Valk et al. 2018).

Furthermore, the genetic findings obtained in this study might be incorporated in the translocation protocol and be used to define the population to where the isolated female muriqui found outside the PNC will be reintroduced (Jerusalinsky et al. 2011).

Future endeavors for improve our understanding and conservation of muriquis in local scales, such as at the Caparaó National Park, should include the implementation of a combined approach of arboreal camera trapping, systematic genetic tagging, and also the assessment of the contemporary genetic diversity in comparison to historic. A better precise estimative and understanding of muriquis populations and their trends over time can be achieved with a systematic and standardized survey by these integrative biomonitoring approaches.

Regarding the muriqui population of the National Caparaó Park, the other five sites not analyzed by both methods (camera trapping and genetic tagging) should be investigated. Further, since our arboreal camera trapping and genetic approaching

demonstrated differences in group size among the sites VA (42 individuals) and VSM (23 individuals), a better investigation in these areas should be conducted to understand whether this difference has been influenced by anthropogenic pressures (e.g. hunting, palm harvesting and illegal logging) or not. Moreover, since two isolated individuals were found outside the park area, the research should be extended and include small fragments surrounding PNC to better understand those individuals/groups living in suboptimal ecological conditions and to manage isolated female muriquis. This recommendation is also supported by the appearance of isolated female muriquis in surrounding other muriqui populations (Tabacow et al., 2009) and other isolated forests (Barros et al., 2011; F.R. Melo, personal comment) in recent years, demonstrating the bias on the muriquis studies toward protected areas and the need to make our effort more applicable to the species conservation.

The later example highlights the importance of increase involvement local stakeholders (i.e. tourists, local community, park managers) in detecting and reporting the occurrence of primates in unexpected areas (Marshall & Wich, 2016). Also, an advantage in evaluating putative research areas might be obtained by the collaboration of park guides whom can enhance the surveillance in the park areas by identifying and reporting locations where muriquis occur within the PNC. This relationship is twofold: the community and park guides contribute by obtaining records of this species and an increased research effort here can contribute towards the management and conservation of this species within protected areas.

In summary, the data provided in this study, improved our knowledge and understanding about the northern muriqui population at Caparaó National Park, which will help to drive and implement conservation actions to ensure species conservation in the long-term. Here, we demonstrated the efficiency and application of two

noninvasive biomonitoring techniques to advancement the research on Neotropical primates and unhabituated northern muriqui populations.

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