University of Venda

Distribution, population status and conservation of the samango monkey (*Cercopithecus albogularis schwarzi*) in the Limpopo Province, South Africa

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Für Mama & Papa

Declaration

I, Birthe Linden, hereby declare that the thesis for the degree of Doctor of Philosophy in Mathematical and Natural Sciences at the University of Venda, hereby submitted by me, has not been previously submitted for a degree at this University or any other University, that it is my own work in design and execution and that all referenced material contained therein has been duly acknowledged.

Signature B. Linden

Date 24th April 2020

Publication timeline and disclaimer

Please note that chapters two to five of this dissertation are stand-alone papers (see below) and a certain degree of repetition was therefore unavoidable. The following chapters are already published, submitted or in preparation for submission for publication in peer reviewed journals:

Chapter 2: Linden B., Foord S., Linden J.D., Taylor P.J. Impact of forest fragmentation on samango monkey (*Cercopithecus albogularis schwarzi*) distribution in a mountain range of northern South Africa. (in preparation)

Chapter 3: Linden B., Dalton D.L., Van Wyk A., Moodley Y., Taylor P.J. Potential drivers of samango monkey (*Cercopithecus albogularis schwarzi*) population subdivision in a highly fragmented mountain landscape in northern South Africa. (submitted to American Journal of Primatology)

Chapter 4: Linden B., Foord S., Horta-Lacueva Q.J.B., Taylor P.J. (2020). Bridging the gap: how to design canopy bridges for arboreal guenons to mitigate road collisions. *Biological Conservation*, 246, 108560.

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Overall abstract

A general introduction about the need for studying forest dwelling primate populations in fragmented landscapes and more specifically in the Soutpansberg is given in **Chapter 1**. Forests affected by fragmentation are at risk of losing primate populations over the long term. In addition, although the impact of fragmentation on primate populations has been studied in many places in Africa, Asia and South America there is no consensus of how the different primate species react to forest disturbance and fragmentation. This study aims to investigate the impacts of natural and anthropogenic forest fragmentation on the Soutpansberg samango monkey population including their distribution, genetics, and phylogeography, identifying threats and mitigation measures. To date, no detailed population level research has been undertaken on samango monkey populations in the far north of their South African distribution. By collecting a diverse data set, in addition to existing ecological data, this study generates conservation and management recommendations suited specifically to the study area and the study species and provides baseline data for future monitoring.

Chapter 2 details how through creating an accurate forest distribution map and collecting samango monkey distribution records through surveys we were able to investigate how both natural and anthropogenic forest fragmentation influences the distribution of samango monkeys in the Soutpansberg. We explored forest patch occupancy and connectivity, determined the degree and nature of matrix utilisation and identified possible threats to forests and samango monkeys between the contrasting landscapes of the eastern and western Soutpansberg. Here we found that samango monkeys largely occupied forest patches <100 ha in size and that the Soutpansberg has very few forest patches > 100 ha available. We showed that samango monkeys used all components of the surrounding matrix and that lone or bachelor group males used the matrix more extensively than groups. We found that paved roads pose a major threat, however not a barrier, to samango monkey populations when navigating the matrix. Forest connectivity was found to be influenced by the distance between patches and possible corridors or stepping stones of isolated forest patches connecting them. Patterns found in this chapter contrasted between the eastern and western landscapes requiring different adaptive strategies from the samango monkeys and different conservation approaches from practitioners. In this chapter we conclude that samango monkeys,

having evolved in fragmented landscapes, are comparatively tolerant and adaptable to a human-transformed matrix.

In **Chapter 3** we focused on the effects that natural and anthropogenic habitat fragmentation in the Soutpansberg has on the genetic diversity and structure of the samango monkey population in the mountain range. Here we used microsatellite and mitochondrial DNA data from faecal and tissue samples collected from four local samango monkey populations across the Soutpansberg. We also included samples from an outlying population on the escarpment south of the mountain, the most likely historical migration route into the Soutpansberg. Our analyses showed that the Soutpansberg population is divided across the mountain and that genetic diversity within the Soutpansberg decreases with increasing distance to the escarpment population and from east to west. We found a lack of contemporary gene flow suggesting that the Soutpansberg population is completely isolated from the closest source population in the escarpment and that populations within the mountain range are isolated from each other. Based on our results we suggest that extensive anthropogenic changes to the landscape in the eastern Soutpansberg and distance between high canopy forest patches in the western Soutpansberg appear to have reduced the ability of samango monkeys to disperse between sampling areas. Overall, we found that natural and anthropogenic fragmentation and geographical distance are potential drivers for the observed population genetic differentiation and that the matrix surrounding forests and its suitability for samango utilisation plays a role at the local scale. In this chapter we conclude that the degree of samango monkey population subdivision and the apparent lack of contemporary migration between populations raises concerns about the long-term viability of populations across the mountain range.

Having identified road fatalities as the main direct anthropogenic threat samango monkeys are exposed to when utilizing the matrix, we further investigated this issue in more detail in **Chapter 4**. Here we aimed to get a better understanding of where roadkills most likely occur and how to mitigate these through using suitable canopy overpasses. As road fatalities threaten primate populations globally, we used the samango monkey (*Cercopithecus albogularis*) as a model species to test the suitability of two different canopy bridge designs through field experimentation and behavioural data collection for arboreal guenon roadkill mitigation. Analysis of actual roadkill data collected in the study area provided insights into the nature of high-risk localities. We showed that canopy overpasses are a viable intervention for mitigating arboreal guenon road fatalities, reducing the probability that monkeys will cross a road on the ground. Samango monkeys clearly preferred a pole bridge over a rope ladder design and canopy bridges were preferred to trees and the ground when the tree canopy was open. Pole bridges were also used by other non-guenon primates and non-primate species. We showed that although samango road kills were not predictable in time (no seasonality), adult female and immature fatalities were predictable in space, restricted to bisected riparian zones and roads close to intact forests. We further found that adult male road fatalities can be expected in seemingly unsuitable habitat areas. This chapter highlights the importance of the correct interpretation of spatial, temporal and demographic data on road fatalities and how experimental research prior to installing crossing structures could increase mitigation impact.

In **Chapter 5** we investigate the regional phylogeography of samango monkeys, providing a deeper understanding into their evolutionary history in repeatedly fragmented forest habitat due to paleoclimatic fluctuations. Here we used mtDNA and microsatellite data obtained from tissue samples from a coastal population (Vamizi Island) in Mozambique and compared this to existing data from South Africa. The additional analysis of Mozambique animals allowed us to further test the number and timing of radiation events of *Cercopithecus* monkeys in southern Africa. In this chapter we propose the occurrence of a single, north-south radiation event during the mid-Pleistocene along the Afromontane forest belt and that after the Last Glacial Maximum, samango populations re-radiated into (re)established coastal forests on a more local scale.

Taking the findings from all chapters together we provide overall conclusions and conservation and management recommendations in **Chapter 6**. Here we also include interview data to give insights into public perceptions of samango monkeys and possible human-primate conflicts as we consider this an important aspect of conservation planning. In this final chapter we conclude that although samango monkeys appear comparatively adaptable to changes in the matrix surrounding their forest habitat, direct threats and forest patch isolation by distance may pose risks to populations in future. We advocate that conservation and management strategies aimed at the long-term persistence of the samango monkey populations and their forest habitat across the

Soutpansberg should be formulated separately for the eastern and western parts of the mountain range as differing landscape variables pose different threats to forest and samango monkeys, thus requiring separate conservation and management approaches. We further recommend explicit mention of distinct management requirements for maternal groups and lone or bachelor group males to achieve a holistic conservation approach for samango monkey populations on the landscape scale. Findings of our study are not only relevant for samango monkey populations in the Soutpansberg but also for populations elsewhere in South Africa and southern Africa generally where forests and samango monkeys share the same paleohistory and current anthropogenic threats.

Chapter 1: The need for studying primate populations in fragmented and anthropogenically transformed landscapes

1. An introduction to primates in fragments

Primates play vital roles in ecosystem structure, functioning and resilience (Estrada et al. 2017). They disperse fruits and seeds; play integral roles in food webs as consumers and prey; and participate in a diverse array of coevolved relationships with other species (Marsh, 2003). Many primate species are frugivorous and play an important part in forest regeneration by dispersing seeds through zoochory (Estrada et al. 2017). Forests suffering from disturbance and/or isolation are at risk of losing their primate populations which in turn could have trickle down effects on plant populations by decreasing genetic heterozygosity and increasing genetic subpopulation differentiation, increasing negative density dependence and decreasing recruitment (Pacheco & Simonetti 2000; Nunez-Iturri et al. 2008; Caughlin et al. 2015).

The impact of habitat fragmentation on primate populations has been studied in many places in Africa, Asia and South America but there is still no consensus as to how primates react to forest disturbance and fragmentation (Marsh 2003). Arroyo-Rodríguez et al. (2013) conducted a review on habitat fragmentation studies involving primates and found that many studies lack the landscape context focusing on single or few fragments only and including only few fragment variables. They showed that while studies investigating the impacts of fragmentation on primate populations are steadily increasing there are still major gaps that need to be filled to reach a more comprehensive understanding. It has further been found that the ability of primate populations to sustain themselves in disturbed and fragmented forests is very species-specific, depending on variation in a range of traits such as dispersal mode, diet and ranging behaviour (Estrada et al. 2017). A species' strategy to cope with fragmentation can further vary between areas of its distribution range depending on site specific circumstances (matrix of surrounding fragments, reasons for fragmentation, additional anthropogenic pressures) (Marsh 2013). As a result, conservation and management recommendations are both species and circumstance specific (Gibbons & Hartcourt 2009). Marsh (2003) suggested that primate species with a low or flexible degree of frugivory, small or variable home range sizes, broad behavioural and dietary plasticity, and the capacity to move through or utilise the surrounding matrix of fragments are most likely to persist in a fragmented landscape.

2. Fragmentation effects on primate populations

Forest fragmentation can have diverse effects on primate populations encompassing molecular, physiological and behavioural processes. In the following section, impacts investigated in this study are introduced.

2.1. Distribution, ranging and matrix utilisation

One of the most profound impacts of fragmentation is a reduction in a species geographic range through local extinctions and subsequent (landscape change induced) prevention of re-colonisation of historical or new suitable areas. If a primate species can sustain itself in a fragmented landscape, it is often required to show some degree of flexibility in its ranging behaviour (Marsh 2003). It is not uncommon that primate species incorporate otherwise unsuitable habitat or even human structures in their daily range or for dispersal between fragments (Carretero-Pinzón 2013). Further, fragments which are unsuitable to permanently sustain a primate population due to their small size can still be utilised for food resources (Carretero-Pinzón 2013). Day ranges and total home range sizes have also been observed to decrease between primate populations in fragmented compared to contiguous forests and often it is not clear what the long-term impact of home range reduction will be (Boonratana 2013; Chalise 2013; Carretero-Pinzón 2013). De la Torre et al. (2013) even observed a change in the ranging mode in pygmy marmosets (*Callithrix pygmaea*) away from a single core area-based home range to a nomadic ranging pattern between patches following fragmentation.

The degree to which primate species are able to utilise the human dominated matrix surrounding fragments also plays an important role in the long-term viability of populations. Here so-called matrix-tolerant species have three main advantages (Laurance 1994): 1) they can disperse between fragments and therefore facilitate gene flow between local populations; 2) they can recolonize fragments following local extinctions; and 3) they often exploit ecological changes in fragments, like edge effects.

2.2. Genetic isolation

Habitat connectivity is a critical consideration for the preservation of forest biodiversity. To ensure long term persistence, many species require dispersal between meta-populations within suitable habitat fragments. Genetically isolated populations may not be viable in the long term, and ecological connectivity allows for dispersal of individuals and genetic material between otherwise isolated sites (Berliner 2005). Small, genetically isolated populations face at least two different kinds of genetic problems (Lacy 1997). The first is increased inbreeding as immigration (and emigration) are impeded. Inbreeding increases the chance of homozygosity of deleterious recessive alleles and their expression and reduces genetic variability which leads to lowered adaptability (Lacy 2000; Bergl et al. 2008; Knapp 2013). Examples of the effect of inbreeding in primates include higher infant mortality (Ralls & Ballou 1982; Noble 1990) and prenatal death (Alberts & Altmann 1995), negative effects on body growth and alteration of the age of first conception (Charpentier et al. 2006) as well as the prevalence of certain diseases and effects on individual longevity (Crawford and O'Rourke 1978; Charpentier et al. 2007). The second problem is an increased vulnerability to genetic drift causing loss of genetic variability through random changes in allele frequencies. Genetic drift and isolation can further lead to outbreeding depression where chromosomal or other mutations acquired in isolation may lead to hybrid infertility when populations come back into contact (Lacy 1997).

2.3. Direct threats

Habitat fragmentation caused by anthropogenic landscape change inevitably results in primate populations having closer contact with humans and their infrastructure (Gordo et al. 2013). Power lines and roads, being a source of mortality, can pose a serious threat to primates when they are forced to move across transformed landscapes (Valladares-Padua 1995; Mass et al. 2011; Teixera et al. 2013; Cibot et al. 2015; Donaldson & Cunneyworth 2015; Kioko et al. 2015; Mclennan & Asiimwe 2016; Al-Razi et al. 2019) (Figure 1). Increased road infrastructure networks bisecting forest patches have further been shown to result in greater access to formerly remote areas which can lead to an increase in poaching (Robinson et al. 1995; Wilkie et al. 2000; Suárez et al. 2009; Laurance et al. 2014).

Proximity to human habitation (e.g. gardens) can lead to injuries or fatalities from domestic dogs (Gordo et al. 2013; Linden et al. 2016) and proximity to agricultural or silvicultural areas often results in human primate conflicts due to crop damage (Droomer 1985; von dem Bussche & van der Zee 1985; Estrada et al. 2017). These conflicts are, more often than not, responded to with fatal control measures such as shooting or poisoning (Chapter 6).

Figure 1. Roadkilled samango monkey in the eastern Soutpansberg.

3. Primates of South Africa

Compared to more tropical areas in Africa primate diversity in South Africa is low with only five species occurring (Linden et al. 2015). Two of these are nocturnal, greater bushbaby (*Otolemur crassicaudatus*) and lesser bushbaby (*Galago moholi*), and three are diurnal, chacma baboon (*Papio ursinus*), vervet monkey (*Chlororcebus pygerythrus*) and samango monkey (*Cercopithecus albogularis*). South Africa's five primates can broadly be divided into savanna- (chacma baboon, vervet and lesser bushbaby) and forest-associated (greater bushbaby and samango monkey) species.

Chacma baboons characteristically inhabit woodland savannas but show a great habitat flexibility making them Africa's most common and widespread primate species (Estes 1991; Skinner & Chimimba 2005). The vervet monkey is the most common and widespread guenon in both South Africa and Africa. It is found in savannas where it is most abundant in and near riparian vegetation (Estes 1991; Skinner & Chimimba 2005). The lesser bushbaby is the most common and widespread southern African representative of the Galagidae and is associated with savanna woodlands, particularly *Acacia* woodland (Estes 1991; Skinner & Chimimba 2005). In contrast, the greater galago and the samango monkey have a much more restricted distribution in South

Africa as they are associated with forested habitats. Whereas the greater bushbaby can also be found in well-developed woodlands and thickets, the samango monkey rarely strays far from high canopy forest habitats, except when in transit or foraging (Skinner & Chimimba 2005). South African samango monkeys represent the southernmost extent of forest guenons in Africa and are the focus of this study (Figure 2).

Figure 2. Female and juvenile samango monkey (*Cercopithecus albogularis schwarzi*) in the western Soutpansberg, South Africa.

4. *The samango monkey (Cercopithecus albogularis)*

When researching a species at the population level (distribution patterns, specific threats, population dynamics and genetics) it is essential to consider as much of the biological understanding as possible (e.g. social structure, ranging behaviour, ecology and taxonomy). Only through the inclusion of species and area specific information can data be comprehensively interpreted and recommendations for conservation and management be given.

4.1. Taxonomy and distribution

The taxonomy of samango monkeys is still disputed on both the species and subspecies level. While some authors consider the samango to fall under the *C. mitis* group (Napier 1981; Grubb et al. 2003; Lawes 2013) others separate them under the species *C. albogularis* (Groves 2001, 2005). Regarding the subspecies, Meester et al. (1986) and Grubb et al. (2003) list two subspecies from the southern African region: *C. a. labiatus* and *C. a. erythrarchus* while Roberts (1951), Dendelot (1974) and Groves (2001, 2005) list an additional third subspecies, namely *C. a. schwarzi*. A recent study by Dalton et al. (2015) (Appendix A.1) of the South African samango subspecies showed that the Soutpansberg and northern escarpment populations are genetically and morphologically distinct from populations further south and suggested a change of the subspecies name from *C. a. erythrarchus* to *C. a. schwarzi* (*sensu* Groves 2001, 2005).

The geographical distribution (notwithstanding the taxonomic debate) of the species ranges from central and eastern to southern Africa where they occur in different evergreen forest types including rainforest, Afromontane and riparian forests as well as swamp and coastal forests (Skinner and Chimimba 2005). They are also found in dry sand forests on the Indian Ocean coast belt in the southern African sub-region (Lawes 1990a, 1992).

The distribution of all South African samango subspecies is closely correlated with the distribution of the main forest types in the country, Afromontane, Scarp and Indian Ocean coastal belt forests. South African forest are characterised by a highly fragmented distribution reflected in the distribution of samango monkey populations (Eeley et al. 1999). Regarding the distribution of the three subspecies, *C. a. labiatus* is endemic to South Africa and range from the Pirie Forest in the Eastern Cape Province north-eastwards to the midlands of the KwaZulu-Natal Province (Hayward et al. 2005), *C. a. erythrarchus* currently occurs from northern KwaZulu-Natal Province, through southern Mpumalanga Province to Zimbabwe, Mozambique and Malawi (Appendix A.2; Linden et al. 2016) although the coastal limits are uncertain (Chapter 5) and *C. a. schwarzi* occurs in Mariepskop (Pilgrims Rest District) north to the Magoebaskloof area including Woodbush (Limpopo Province) (Roberts 1951; Groves 2001) as well as the Soutpansberg (Appendix A.1; Dalton et al. 2015). Whether or not the subspecies *schwarzi* is endemic to South Africa remains unclear until more genetic data become available from populations further north (e.g. Eastern Highlands of Zimbabwe) (Appendix A.2; Linden et al. 2016).

In the Limpopo Province sizeable patches of Afromontane forest habitat are located along the escarpment and the Soutpansberg and Blouberg ranges. Many of these patches support populations of samango monkeys whose distribution and ecology have been little studied, especially at the population level, in comparison to *C. a. labiatus* and *C. a. erythrarchus* populations further south (Lawes 1990a, 1990b, 1991, 1992; Lawes & Piper 1992; Lawes et al. 1990; Lawes et al. 2000; Madisha et al. 2017; Wimberger et al. 2017). The escarpment and Soutpansberg support the two major samango populations in Limpopo Province and given the extent of unsuitable habitat between these geographic features it is unknown to what degree populations are connected (Chapter 3). No evidence, published or anecdotal, exists about the occurrence of samangos in the Blouberg inselberg, although a group of samangos has recently been recorded from the isolated and semi-arid Makgabeng plateau, apparently inhabiting riparian forests (Stuart & Stuart 2007) (Chapter 2).

4.2. Conservation status

The most recent Red List of Mammals of South Africa, Swaziland and Lesotho includes the subspecies *schwarzi* following recommendations by Dalton et al. (2015) and assessed all three samango subspecies separately (Linden et al. 2016). Since 2016 the subspecies *C. a. labiatus* is listed as Vulnerable, *C. a. erythrarchus* as Near Threatened and *C. a. schwarzi* as Endangered. Assessments were based on inferring the area of occupancy using the amount of remaining natural habitat within forest patches greater than 1.5 km^2 in extent (below which forest patches are generally unoccupied by samangos, Lawes 1992). For all three subspecies, a continuing decline in the area of occupancy is assumed due to ongoing forest habitat loss across the country, a suspected continuing decline in habitat quality from commercial forestry, and a suspected continuing decline in mature individuals from frequent reports of mortality from road collisions, electrocutions, snaring and hunting for traditional medicine (Linden et al 2016). The previous Red List assessment (Friedman & Daly 2004) included *C. a. schwarzi* as a synonym of *C.a. erythrarchus* listed as Vulnerable and *C. a. labiatus* as Endangered. Globally, the IUCN has not yet listed *C. albogularis* as a separate taxon, placing the samango monkey under *Cercopithecus mitis* which is listed as Least Concern (ver. 3.1) with an overall decreasing population trend (Kingdon et al. 2008). The

IUCN lists the subspecies *erythrarchus* as globally Least Concern (Kingdon & Lawes 2008) and the subspecies *labiatus* as globally Vulnerable (Lawes 2008) and has not assessed the subspecies *schwarzi* separately from *erythrarchus*. Global Red List assessments are therefore in need of updating.

4.3. Diet

Samango monkeys are known to utilise a wide variety of forest plants with a significant part of their diet comprising fruit (52% in KwaZulu Natal, Lawes et al. 1990; 72% in Limpopo Province, Linden et al. 2015). In the Soutpansberg they were found to disperse the seeds of 22 plant species via defecation, cheek pouch carrying and spitting or dropping, indicating that they play an important role in forest plant dynamics (Linden et al. 2015). Furthermore, through their foraging behaviour samango monkeys were found to make fruit available to ground dwelling mammals such as bush pig (*Potamochoerus porcus*), red duiker (*Cephalophus natalensis*) and porcupine (*Hystrix africaeaustralis*) which may act as subsequent seed dispersers (Seufert et al. 2009). Nowak et al. (2016) and Wimberger et al. (2017) found that samangos show adaptability to utilising exotic (two species of oak trees: *Quercus robur* and *Q. palustris*) and invasive plant species (black wattle: *Acacia mearnsii*) in anthropogenically transformed landscapes.

4.4. Social structure, group size and ranging behaviour

Samango monkeys are philopatric and live in multifemale groups being led by a single adult male (Cords 2001). They are male dispersed and males are evicted from their maternal group approximately a year before reaching sexual maturity (Henzi & Lawes 1987). Tenure of the presiding male lasts between 5 months and 3.5 years due to regular contestation by outside males during annual breeding seasons (Butynski 1990). Single males or bachelor groups not associated with groups of females are therefore a common sight and it is not unusual for these males to "sneak mate" with group females during breeding season (Henzi & Lawes 1987; Macleod et al. 2002; Roberts & Cords 2015). These males are described to range widely and interact with several groups of females (Swart & Lawes 1996).

Both group and home range sizes vary across study sites in South Africa with groups consisting of between 16 to 60 individuals (Lawes 1990a; Lawes 1992; Coleman & Hill 2014; Novak et al. 2014; Linden et al. 2015; Wimberger et al. 2017) and home ranges extending from 15 to 54 ha

(Lawes et al. 1990; Coleman & Hill 2014; Wimberger et al. 2017). Home ranges of neighbouring groups often overlap (Lawes 1992; Lawes & Henzi 1995; Novak et al. 2014) and extra-group male ranges can overlap considerably with group home ranges (Swart et al. 1993).

Although there are no data on the life-history of wild samango monkeys, a 30 year long study on wild blue monkeys (*Cercopithecus mitis stuhlmanni*) in Kenya found that females give birth for the first time at the age of 7 years on average and that a new infant is born after 2-3 years if the previous one survives the first year (Cords 2012). The study also found that the oldest female lived to at least 33.5 years and several others lived into their 20's. The oldest recorded female was observed not to have offspring in the last 11 years of her life. Such detailed life-history data are not available for wild males due to the difficulties of tracking them after they leave the natal group.

5. *The Soutpansberg*

This study will assess the distribution, population status and conservation of the Soutpansberg samango monkey populations with reference to adjacent forested areas including the Blouberg inselberg and Makgabeng cuesta in the west (Chapter 2) and the northern escarpment to the south (Chapter 3) of the study area (Figure 3). The Soutpansberg is located within the Vhembe Biosphere Reserve (Pool-Stanvliet 2013) in South Africa's far northern Limpopo Province.

The Soutpansberg is an east-west orientated mountain range varying in altitude from 200 m near Pafuri in the east to 1 748 m (Lejuma) in the west (Hahn 2017a). It is ~ 210 km long and ~ 60 km wide (widest point) and is characterised by extreme north-south as well as east-west rainfall gradients driven by altitude and aspect (Hahn 2011). The average annual rainfall, typically received during summer between October and April, decreases from south (618 mm, Louis Trichardt) to north (367 mm, Waterpoort) and east (1 874 mm, Entabeni) to west (462 mm, Mara/Vivo) (Hahn 2017a). The prevailing winds in the Soutpansberg come from the east-south-east causing moisture laden air from the Indian Ocean to condense on the southern slopes resulting in orographic rain and fog precipitation while creating a rain shadow on the northern slopes.

The Soutpansberg is a recognised Centre of Plant Endemism (CPE) with 22 strict endemics although this rises to 40 taxa if the Blouberg and Makgabeng are included (Van Wyk & Smith 2001; Hahn 2017a) and centre of floristic diversity with 2 443 vascular plant taxa belonging to 922 genera in 187 families representing 74.2% of southern African vascular plant families and 48.8% of the genera within an area of only 6 700 km^2 (Hahn 2019).

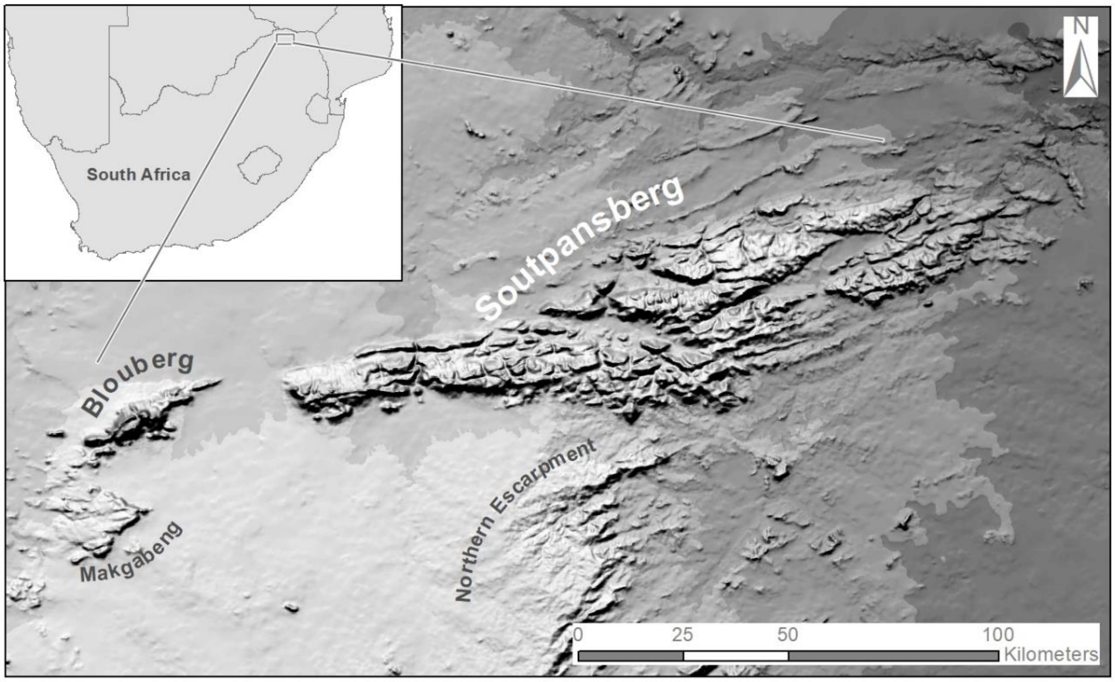

Figure 3: Location of the Soutpansberg mountain range, Blouberg inselberg, Makgabeng cuesta and northern escarpment in Limpopo Province, South Africa.

6. Human history and landscape change in the Soutpansberg

The Soutpansberg has a long history of human settlement and activity including various cultural groups (later united as the VhaVenda) starting from about AD 300 onwards with Bantu speaking farmers settling alongside rivers in the mountain range (Eastwood & Eastwood 2006). One of the earliest Iron Age sites (AD 350) found in the Soutpansberg is at Happy Rest (Schoemansdal) showing evidence of livestock herding (sheep and to a lesser extent cattle) (Voigt & Plug 1984). Owning of livestock, particularly cattle, became of great importance to early Soutpansbergers and by the 19th Century cattle were considered more valuable than iron implements (Voigt 1983). Evidence of vegetation change coinciding with the first settlers in the area exists. Scott (1987) showed a sharp decline in tree pollen at around 1500 BP and he suggested that this might be linked to the burning and clearing of woody vegetation by the first Iron Age people in the area. The establishment of a more formal Venda state in the area took place between AD 1688 and 1800 (Tempelhoff 1999). The first people of European descent arrived in the area from the 1820's onwards and some of their early activities included the trade of wildlife products (ivory, horns, skins, meat) and timber from local forests (Tempelhoff 1999). As the demand for timber increased, fast-growing exotic timber plantations were established from the 1890's and grew rapidly from the 1920's onwards (Scholes et al. 1995). In the Soutpansberg, these commercial timber plantations were mainly established in grasslands adjacent to indigenous forests on the southern slopes of the wetter, eastern parts of the mountain (Geldenhuys 1997). Large scale agricultural practices in the eastern Soutpansberg, particularly in the fertile Levuvhu area, started from the late 1930's (Tempelhoff 1999). Hahn (2017b) showed a rapid demise of the Soutpansberg grasslands coinciding with the onset of commercial agriculture and silviculture which forever changed the more open landscape of forest-grassland mosaic in the Soutpansberg. Figure 4 shows the current extent of anthropogenic land transformation across the Soutpansberg.

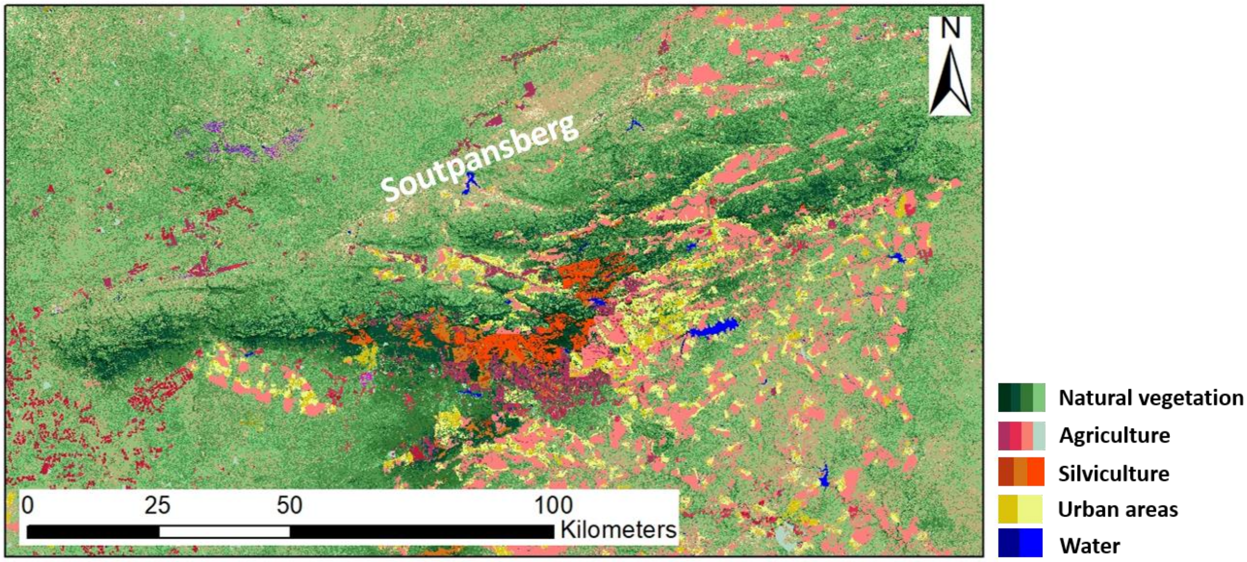

Figure 4. Land transformation across the Soutpansberg mountain range. Map data: South African national land-cover (NLC) (2014).

7. *The state of indigenous forests*

7.1. South Africa

South African forests occur scattered along the Great Escarpment, mountain ranges, and coastal lowlands in small to very small patches, most of them < 100 ha in size (Cooper 1985; Geldenhuys 1991). Indigenous forest covers less than 0.4% of the surface area of South Africa and has the highest biodiversity per unit area of any biome in the country with 3 000 species in ~ 5 052 km^2 as opposed to the next highest, fynbos, with ~ 7 500 species in 76 744 km^2 (Berliner 2005). Internationally, South African forests can also claim global importance. Despite sharing more affinities with Afrotropical forests, their position relative to the equator qualifies them as ‘temperate forests’. Research has shown that South African forests have the highest biodiversity of any temperate forested region in the world (Silander 2001). They are between three and seven times richer in tree species than other forested areas of the Southern Hemisphere, even though these forests cover a much larger area. Furthermore, when it comes to the richness of genera and families of trees, South African forests are unparalleled (Cowling 2002; Silander 2001). In theory, all South African forests are protected under the National Forests Act (Act No 84 of 1998), but in practice considerable legal and illegal exploitation is occurring and the conservation status of much indigenous forest is uncertain (Castley & Kerley 1996; Lawes et al. 2001).

7.2. Limpopo Province

The Limpopo Province has, with 5%, one of the smallest shares of national forest cover with an estimated area of 192 km^2 (Berliner & Benn 2004), occurring mostly along the Blouberg, Soutpansberg and northern escarpment. Geldenhuys and Mucina (2006) classified the forests of the Soutpansberg and part of the Magoebaskloof area of the escarpment as Limpopo Mistbelt Forest within the Northern Mistbelt Forest group (which includes forest communities along the Mpumalanga Province escarpment) classified by Von Maltitz et al. (2003). The Soutpansberg, in particular has been identified as a refugium for these forests (Geldenhuys & Mucina 2006) which are characterised by considerable biodiversity (Hahn 2019) and provide habitat for many rare and endangered taxa including the samango monkey (Linden et al. 2016).

Despite having been identified by the Department of Water Affairs and Forestry (DWAF) as 100% irreplaceable, Northern Mistbelt Forest has one of the lowest levels (< 5%) of this category under strict protection. A gap analysis has shown that more than 70% of the conservation target areas for this vegetation type are outstanding (Berliner 2005). There is little quantitative data on current threats to the forests of the Limpopo Province. However, non-sustainable harvesting by rural communities for fuelwood, building materials and medicinal plants is on the increase and forests under particular pressure include areas in Limpopo (Berliner 2005). Forests of the Limpopo Province are variously state-owned, privately owned or community owned.

7.3. Soutpansberg

Forests of the Soutpansberg are naturally highly fragmented and restricted owing to the complex topographical and climatic patterns of the mountain range (Chapter 2). The natural forest fragmentation has been exacerbated by more recent anthropogenic activities especially in the eastern parts where expansion of timber plantations between 1990 to 2006, wood collection and settlement expansion are considered significant reasons for an observed reduction in forest and woodland cover (Munyati & Kabanda 2008). Subsistence farming, timber (*Pinus* spp. and *Eucalyptus* spp.) plantations and sub-tropical fruit orchards are further fragmenting and/or isolating forests, particularly in the eastern, higher annual rainfall areas of the mountain range (Figure 5). Forests in the Soutpansberg are driven by shade effects and runoff from cliffs, rainfall, fog precipitation and availability of groundwater and as a result of the varying fog and rain precipitation patterns across the Soutpansberg, high canopy, evergreen forests suitable for samango monkeys are only found on the wetter southern ridges of the mountain and individual forest patches tend to be fewer and smaller in the western parts compared to the eastern parts (Chapter 2).

Figure 5. Examples of the matrix surrounding forest patches in the eastern Soutpansberg. Picture a shows forest fragments in a landscape of a recently cleared timber plantation for pulp production. Picture b shows fruit orchards in the foreground directly bordering timber plantations. Only the steep inaccessible slopes are still covered by indigenous forest.

8. Rationale of the study

This study aims to investigate the impacts of natural and anthropogenic forest fragmentation on the samango monkey populations of the Soutpansberg including distribution, population genetics, threats and mitigation, and phylogeography. To date, no detailed population level research has been undertaken on samango monkey populations in the far northern range of their South African distribution. Given that the subspecies in the Soutpansberg has been listed nationally as Endangered and that populations in the mountain range are most likely geographically isolated (Linden et al. 2016) there is an urgent need for a more detailed population assessment to ensure their long-term persistence. This is given impetus by the fact that more than 95% of Limpopo forests, including their fauna, are not under strict protection (Berliner 2005).

9. Aims and objectives of this study

By collecting a diverse data set in addition to existing ecological data this study aims to generate conservation and management recommendations suited specifically to the study area and the study species. Results presented here may also act as a starting point for population monitoring to assess population trends going into the future. In the following the aims and objectives of each chapter are outlined in more detail.

Chapter 2 focuses primarily on indigenous forest and samango monkey distribution across the Soutpansberg. Forest distribution and fragment characteristics across the Soutpansberg were analysed using a Geographical Information System (GIS), ground truthing as well as historical, aerial photographs. As no adequate forest distribution mapping for the Soutpansberg exists this chapter provides the first detailed spatial analysis of this habitat type to assess the extent of available forest habitat for samango monkeys. Using the distribution of forests as a basis, a long-term distribution survey was undertaken to establish the current extent of samango distribution across the Soutpansberg. This was done through direct forest surveys and public participation. Both spatial data sets were then analysed to establish correlations between them. Consideration is also given to the possible distribution of samango monkeys in two upland areas west of the Soutpansberg, the Blouberg inselberg and the Makgabeng cuesta.

In **Chapter 3** the population genetic processes of samango monkeys in the Soutpansberg are investigated to understand impacts that historical and recent forest fragmentation and landscape change might have on the populations. Here microsatellite data were analysed to investigate population subdivision, genetic diversity of sub-populations, the degree of inbreeding within populations and gene flow (migration rates) between them. Molecular results were contextualised with landscape variables (geographic barriers, natural and anthropogenic fragmentation) to identify possible drivers for population subdivision.

In **Chapter 4** one of the main direct threats to samango monkeys identified in the study area, road fatalities, was closely investigated and mitigation measures tested. Road crossings and samango-car collisions were recorded opportunistically through public participation and data were analysed spatially with a GIS. Using the actual road crossing and mortality data as a basis, an experimental study was undertaken to test suitable canopy overpasses to mitigate samango monkey road fatalities. The experiment included recording of samango monkey behaviour when crossing two different canopy bridge designs under three different canopy cover scenarios.

Chapter 5 uses existing and new molecular data to shed more light on the phylogeography of *Cercopithecus* monkeys in southern Africa. By analysing new microsatellite and mtDNA data from a samango monkey population in Mozambique this chapter aims to further test the number and timing of radiation events of *Cercopithecus* monkeys in southern Africa and extends recent work on this topic published in 2015 (Appendix 1).

Lastly, **Chapter 6** provides a synthesis of the findings and includes questionnaire data to provide recommendations of how these can be integrated in management and conservation plans for the species.

Appendix 1 and 2 include abstracts of peer-reviewed publications that are an outcome of the work presented here. **Appendix 1** presents a paper abstract from a collaborative study on samango monkey speciation which discusses the South African subspecies. **Appendix 2** presents the abstract of the most recent Red List assessment of the samango monkey in South Africa.

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Chapter 2: Impact of forest fragmentation on the distribution of an isolated samango monkey (*Cercopithecus albogularis schwarzi*) population in the Soutpansberg mountains, South Africa

Abstract

Habitat fragmentation is typically a landscape-scale, human-induced process whereby contiguous habitat is separated into smaller pieces with overall habitat area loss leaving isolated fragments embedded in a human dominated matrix. Paleoclimatic change or other natural events also give rise to fragmented patches of habitat, creating natural mosaic landscapes. The important difference between the two fragmentation drivers is the time scale. Deforestation continues at rates unprecedented in evolutionary history, making forests and their fauna particularly vulnerable to habitat fragmentation. Forest fragmentation affects the viability of primate populations worldwide but there is still no general understanding of primate responses to forest fragmentation. Here we investigate how both natural and anthropogenic forest fragmentation influence the distribution of samango monkeys in the Soutpansberg in northern South Africa by exploring forest patch occupancy and connectivity, determining the degree and nature of matrix utilisation and identifying possible threats to forests and samango monkeys between the contrasting landscapes of the east and west of the mountain range. By creating an accurate forest distribution map and collecting samango monkey distribution records through surveys we found that they largely occupied forest patches < 100 ha in size and that the Soutpansberg has very few forest patches > 100 ha available. Our results show that samango monkeys used all components of the surrounding matrix and that lone or bachelor group males used the matrix more extensively than maternal groups. We found that paved roads pose a major threat to samango monkey populations when navigating the matrix. Forest connectivity was found to be influenced by the distance between patches and possible corridors or stepping stones of isolated forest patches connecting them. Patterns found in this study differ between the eastern and western landscapes requiring different adaptive strategies from the samango monkeys and different conservation approaches from practitioners. Our results support suggestions that, having evolved in fragmented landscapes, samango monkeys are comparatively tolerant and adaptable to a human-transformed matrix.

1. Introduction

Habitat fragmentation is typically a landscape-scale, human-induced process in which contiguous habitat is separated into smaller pieces resulting in overall habitat area loss and leaving isolated fragments embedded in an anthropogenically modified landscape (the matrix) (Fahrig 1999; Fahrig 2003). Major drivers of habitat fragmentation and loss are urban sprawl, expanding agricultural land and forestry industries (Bierregaard et al. 1992; Laurence et al. 2002). Habitat fragmentation and loss are key reasons for global biodiversity loss (Rands et al. 2010).

Paleoclimatic changes or other natural events (e.g. fires) can also give rise to fragmented patches of habitat, creating natural mosaic landscapes (Rogan & Lacher 2018). The important difference between natural and anthropogenic fragmentation is the time scale involved. Anthropogenic habitat fragmentation is characterised by rapid change at rates that far exceed the adaptive capacity of most species (Skole & Tucker 1993; Pimm et al. 1995; Myers & Knoll 2001), while the long-term nature of change in natural mosaic landscapes allows for adaptive responses to patchiness over evolutionary timescales (e.g. through traits that confer increased dispersal ability) (Didham 2010). With an estimated annual forest loss of 5 Mha year–1 (Curtis et al. 2018), deforestation continues at alarming rates, unprecedented in evolutionary history, making the world’s forests particularly vulnerable to anthropogenic habitat fragmentation (Bierregaard et al. 1992).

Primates are iconic components of Africa’s diverse forest mammals, forming part of numerous co-evolved relationships with other taxa and providing key ecosystem services such as seed dispersal (Marsh 2003; Linden et al. 2015; Estrada et al. 2017). Fragmentation of forests affects the viability of primate populations worldwide and forest dwelling primates are impacted more strongly by habitat loss than those living in savannahs (Johns & Skorupa 1987; Cowlishaw & Dunbar 2000). Although the effects of habitat fragmentation on primate populations have been studied throughout all continents they are found, there is still no general understanding of primate responses to forest disturbance and fragmentation (Marsh 2003). Predictions are often confounded by the varying nature of matrices surrounding fragments (Marsh 2013).

South Africa is home to only one diurnal forest dwelling primate species, the samango monkey (*Cercopithecus albogularis*). Both indigenous forests and samango monkeys are of high conservation concern in the country as the distribution of both is very patchy and restricted, with forests covering less than 0.4% of the countries land surface area (Berliner & Benn 2004).

Here we investigate how habitat fragmentation influences the distribution of samango monkeys in an isolated mountain range, the Soutpansberg, in far northern South Africa. Indigenous forests in the Soutpansberg are both naturally (Eeley et al. 1999) and anthropogenically fragmented (Scott 1987; Munyati & Kabanda 2008; Griscom et al. 2010) representing two main matrix scenarios. The matrix in the eastern part of the mountain range is characterised by sub-tropical fruit orchards, commercial forestry (silviculture), subsistence agriculture and urban settlements while that in the western more arid region largely comprises a novel vegetation type of indigenous species (Hahn 2017a), classified as Soutpansberg Mountain Bushveld (Mucina & Rutherford 2006). This woodland/thicket vegetation type is shown to be the result of bush-encroachment, largely driven by anthropogenic practices (including alteration of fire regimes, extirpation of large herbivores, and increasing atmospheric CO_2 levels; O'Connor et al. 2014) replacing grasslands across the southern aspects of the mountain range (Hahn 2017a) and resulting in less defined forest boundaries than in the historical forest-grassland mosaic. Currently, no accurate fine scale map of forest distribution exists for the study area and no detailed samango monkey distribution data across forest patches is available. Knowledge of a species’ distribution is vital (Ramos-Fernandez et al. 2013) to formulate suitable conservation strategies, including the delineation of protected areas, the management of unprotected habitats and the maintenance or creation of corridors between habitat fragments (Margules & Pressey 2000; Margules & Sarkar 2007).

Patch occupancy, matrix utilization and mortality risks in human dominated landscapes are central to assessing the long-term viability of primate populations (Marsh 2013). By creating a fine-scale distribution map of indigenous forests and using samango monkey distribution data we aim to explore forest patch occupancy and connectivity, determine the degree and nature of matrix utilisation, and explore possible threats to forests and samango monkeys in the contrasting landscapes associated with the eastern and western parts of the Soutpansberg.

2. Materials & Methods

2.1. Study area

The study was conducted in the Soutpansberg mountain range as well as two outlying areas, the Blouberg inselberg and the Makgabeng cuesta, in the Limpopo Province of South Africa (Figure 1). While the main focus of the study was on the Soutpansberg samango monkey populations, the two outlying areas were included as there was no clarity on the presence of samango monkeys there. Although the Blouberg contains indigenous high canopy, evergreen forest habitat suitable for the samango monkey neither historical nor contemporary data exist on their presence. The isolated, semi-arid Makgabeng contains riverine forest along the seasonal Masebe River which would be considered marginal for samango monkey occurrence although there is one record of occurrence (Stuart & Stuart 2007).

The Soutpansberg is characterised by extreme north-south as well as east-west climatic gradients driven by altitude and aspect (Hahn 2011). The average annual rainfall decreases from east (1 874 mm, Entabeni) to west (462 mm, Mara/Vivo) and south (618 mm, Louis Trichardt) to north (367 mm, Waterpoort) (Hahn 2017b). Prevailing winds from the east and south-east causes moisture laden air to rise over the southern slopes resulting not only in rain but also precipitation from fog. Due to this, high canopy, evergreen forests suitable for samango monkeys are generally only found on the wetter southern ridges of the mountain. Precipitation from cloud immersion plays a major role in sustaining these Afromontane forests, hence their classification as Limpopo Mistbelt Forests (Von Maltitz et al. 2003; Geldenhuys & Mucina 2006). Although this forest type was identified as 100% irreplaceable by the then Department of Water Affairs and Forestry (DWAF), it has one of the lowest levels (< 5%) of this category under strict protection (Berliner 2005).

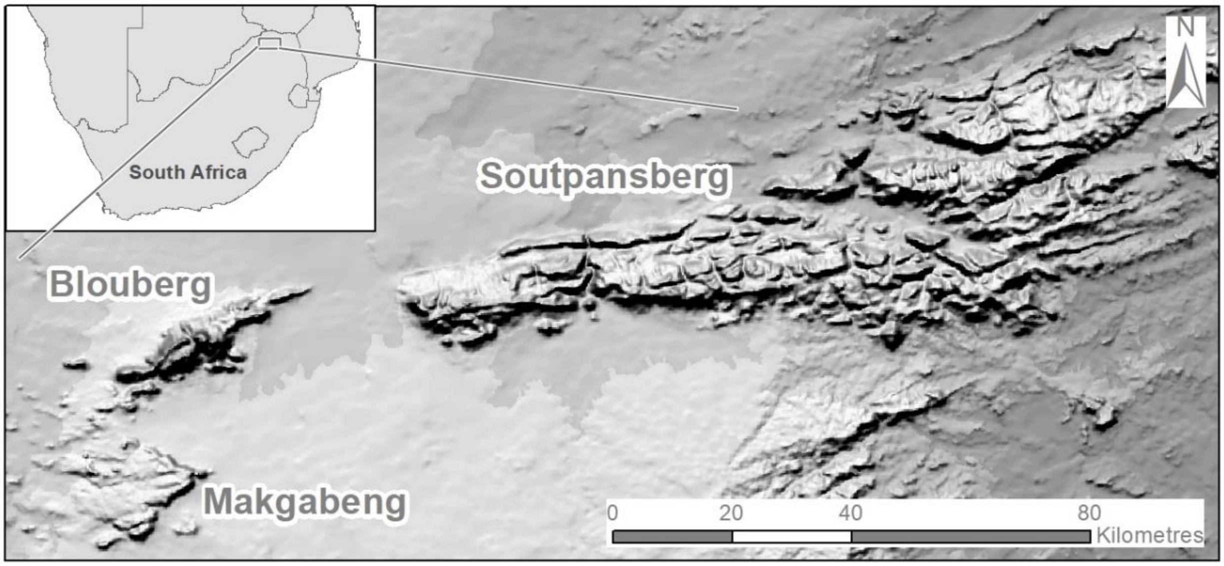

Figure 1. Study area showing the Soutpansberg mountains, Blouberg inselberg and the Makgabeng cuesta in far northern Limpopo Province, South Africa.

2.2. Study species

The samango monkey (*Cercopithecus albogularis*) is South Africa's only exclusively forest dwelling, diurnal primate species and represents the southernmost extent of the range of arboreal guenons in Africa. The distribution of samango monkeys in South Africa is closely correlated with that of Afromontane, Coastal and Scarp forests. This study focuses on one of the three samango monkey subspecies occurring in South Africa, *C. a. schwarzi* (Dalton et al. 2015) which is nationally listed as Endangered (Linden et al. 2016).

Samango monkeys live in multi female groups of between 16 to 60 individuals (Lawes 1990; Lawes 1992; Coleman & Hill 2014; Novak et al. 2014; Linden et al. 2015; Wimberger et al. 2017) with a single adult male who is contested by outside males annually during breeding season (Butynski 1990; Cords 2001). While female samango monkeys are philopatric, males are evicted from their maternal group when reaching sexual maturity (Henzi & Lawes 1987). Lone or bachelor group males are common in samango monkeys and are described to range widely, interacting with several groups of females (Swart & Lawes 1996). Home range sizes vary across study sites in South Africa extending from 15 to 54 ha (Lawes et al. 1990; Coleman & Hill 2014; Wimberger et al. 2017) with neighbouring groups often overlapping (Lawes 1992; Lawes & Henzi 1995; Novak et al. 2014). Lawes (1992) suggested that they did not occupy forest patches < 100 ha in KwaZulu Natal.

2.3. Delineation of forest

Spectral confusion can make distinguishing evergreen indigenous forest from non-forest difficult in image classification, particularly with adjacent thicket and woodland (Berliner 2005) with one study in the survey area reporting only 46% accuracy in classifying indigenous forest (Griscom et al. 2010). None of the existing national forest layer datasets (raster datasets from classification of satellite imagery) were considered accurate enough at the scale desired for this study. This became clear when examining the 2013-2014 and 2018 national land-cover (NLC) datasets (Department of Environmental Affairs 2015; Department of Environmental Affairs 2018) of 30 m and 20 m resolution respectively and the 2018 national vegetation map based on the latter (South African National Biodiversity Institute 2006-2018). We found these datasets land cover classes to be unsuitable and in cases erroneous at the scale of the study including non-forested areas and excluding patches of high canopy indigenous forest visited in this study.

To create a more refined and accurate forest distribution layer for the Soutpansberg we mapped forest patches in ArcGIS 10.5 (Esri®) making use of various methodologies and resources. All mapping was done using the South African coordinate reference system and as the area of forests distribution fell between 22° 29' 30" E and 30° 25' 40" E (with different longitudes of origin between the east and west survey areas), Hartebeesthoek94 Lo31° was used for the eastern part and Hartebeesthoek94 Lo29° for the west.

For the base map we mosaicked 2008 orthorectified true colour aerial imagery available from National Geo-spatial Information (http://www.ngi.gov.za/; Department of Rural Development and Land Reform, Republic of South Africa). These images have a ground sample distance of 0.5 m and orthorectification accuracy of approximately 3 m. Indigenous forests were determined in the 2008 aerial photography by heterogeneity of canopy crowns, closed canopy cover, colour and shadow (e.g. Cooper and Smith 1966) and ground truthing during samango monkey distribution surveys. Afromontane, riparian and regrowth forest were not distinguished.

Additionally, Landsat 8 enhanced vegetation index (EVI) Tier 1 32-day composites (30 m resolution) were downloaded from Google Earth Engine (https://earthengine.google.com; Landsat Spectral Indices products courtesy of the U.S. Geological Survey Earth Resources Observation and Science Center) and overlaid on the aerial photography to assist in identifying forest patches. EVI is seen as an optimized product against the normalised difference vegetation index (NDVI), particularly in areas of high biomass, also in responsiveness to canopy structure and with a reduction in atmospheric influences (Huete et al. 2002). Composites were chosen to be representative of the driest time of year (August and September in the years 2016-2019) as the evergreen forest of the study area is highly distinguishable from adjacent semi-deciduous woodland and thicket when viewed in imagery selected only from the late dry season. Individual forest patches were delineated manually at a scale of 1:1 500 or less and were based on continuous connectivity.

In comparison to the west, extreme forest fragmentation and extensive land use in the eastern Soutpansberg made identification and mapping of indigenous forests more challenging. Here, eastern Soutpansberg land use maps (vector data) available from Komatiland Forests (South African Forestry Company Limited) for the Entabeni and Thathe Vondo state forests delineating indigenous forest (amongst other classes) were used as a basis for refinement of the forest layer for those areas. Each polygon was examined and included when suitable with delineation modified as necessary to exclude open areas, woodland or portions of timber plantations or to include indigenous forest judged to be erroneously assigned in another class.

Paved roads, although not a barrier to samango monkeys (Linden et al. 2016; Linden et al. 2020), were considered a substantial forest edge and forest patches were separated when these bisected them.

2.4. Samango monkey distribution survey

The distribution survey was conducted from 2011 to 2019. Here we distinguished between maternal groups and single or bachelor group males for all distribution records. As the study area was comparatively large we included the general public through regularly placing articles in two local newspapers asking people to report samango monkey sightings in the greater Soutpansberg area. People were asked to report observations and, if possible, take a picture of the samango(s) and take a GPS point or Google Earth placemark of the sightings. We further requested to be contacted so that we could verify the report by visiting the site and confirming correct identification though direct observations.

In addition to the public distribution records we visited forest patches across the mountain range for direct surveys to establish samango monkey presence or absence and ground-truth forest delineation. The presence of samango monkeys in a forest can be confirmed either through actual sightings or through their distinct vocalisations. Samango monkey males use two characteristic calls, the “boom” and the “pyow”, which are most frequently heard at dusk and dawn when they are most active (Lawes & Piper 1992). There is further evidence that lone and bachelor males do not emit the “boom” call (Linden pers. obs.). We surveyed a total of 32 forest patches for a minimum of two and maximum of four days, always with two observers present. This period was deemed suitable for establishing samango monkey occurrence in a forest patch as the described vocalisations can be heard regularly if monkeys are present. When surveying a forest patch, we slowly walked along game trails or existing human paths and regularly used vantage points such as cliffs from which we scanned the forest canopy with binoculars and listened for vocalisations. When we heard samango monkeys we attempted to get closer to them to confirm if it was lone or bachelor group males or maternal groups. We confirmed absence in two and presence in 30 of the 32 surveyed forest patches.

As forests are variously state, private or community owned, relevant permission was sought either from the landowners, community authorities or state entities (Komatiland Forests, Licence Number: 19017 and Licence Number: 19023).

2.5. Analysis

Spatial statistics including the total area (ha) and perimeter (m) of each indigenous forest patch were done in ArcGIS 10.5 (Esri®) using the extension Patch Analyst version 5.2.0.16 (Rempel et al. 2012). The confirmed area of occupancy (AOO) for samango monkeys was determined from the samango monkey distribution points and the total area of suitable forest habitat mapped, where AOO equalled the area of all forest patches with confirmed samango monkey presence. In this study, forest patch occupancy was equivalent to the presence of samango monkey groups as we never encountered males without groups in forests, likely due to the difficulties of detecting single or bachelor groups males inside a forest.

To determine matrix utilisation, Euclidean distance of samango monkey distribution records located in the matrix to each nearest suitable forest patch was calculated using the ArcGIS proximity toolbox. This enabled us to infer the maximum dispersal distances and also infer forest patch connectivity.

Land tenure of forest patches or parts thereof was classed as private ownership, community ownership and state ownership determined through cadastral layers (Chief Surveyor-General, Republic of South Africa) and local government ownership databases (Makhado Local Municipality general valuation roll for the period 2014 to 2018). Protected area status of forest patches or parts thereof were determined by using the 2019 South Africa Protected Areas Database (Department of Environmental Affairs 2020).

The amount of edge exposure of a forest patch to influences from the outside matrix is largely dependent on the size and shape of the patch. Here, most measures of patch shape focus on some variation of the perimeter to area ratio (Krummel et al. 1987). The shape index (SI) is the perimeter (P) per unit area (A) of a fragment, where SI = P/A and where complex shapes have a larger perimeter (or edge) for a given area resulting in a higher perimeter: area ratio. Here, we calculated the SI using Patch Analyst version 5.2.0.16 (Rempel et al. 2012). Shape index values > 0 indicate increased deviation from a circular shape.

$$SI = \frac{P}{\sqrt{4\text{л}A}}$$

We further calculated relative rankings of forest patches using the shape index values derived from the above formula. Values were scaled into 1–0 rating where a forest that is very narrow and indented will have a scaled shape index that approaches 1, while a forest patch that is close to circular in shape will have a scaled shape index that approaches 0. This was done using the following scaling formula (e.g. Berliner 2005):

$$1 - \frac{(highest\ SI - actual\ SI)}{highest\ SI}$$

As the eastern and western Soutpansberg have very different drivers regarding forest distribution (rainfall patterns, contemporary fragmentation) shape indices were initially calculated and scaled separately to allow for the detection of different patterns in those two landscapes.

3. Results

3.1. Forest patches

The survey area was 109 km east to west with a 21 km gap between east and west based on mapped forest fragments (Figure 2). Forests ranged from an altitude of 650 m in the east to 1 650 m in the west with a general increase in altitude of forest patches from east to west. Forests were predominantly located on the southern and eastern aspects of the mountains. Altitude and aspect were determined using the NASA Shuttle Radar Topography Mission (SRTM) digital elevation model at a 30 m resolution (Figure S.1). The refined mapping of forests in the study area identified 233 suitable indigenous forest patches for samango monkeys, covering an area of 9 285 ha across the Soutpansberg. Here, 202 suitable forest patches covering 8 375 ha were identified in the east and 31 suitable forest patches covering 910 ha in the west (Figure 2). Forest patches ranged in size from 1 ha to 3 762 ha (Table S.1).

The average SI for forest patches in the western Soutpansberg was 3.4 (range = 1.45 – 7.21) with an average scaled SI of 0.47. For forest patches in the eastern Soutpansberg we found a lower average SI of 2. with greater variation (range = 1.1 – 16.3) and an average scaled SI of 0.17.

National Land Cover (NLC) 2013-2014 and 2018 datasets were clipped to the survey area and extent (hectares) calculated. In comparison to our forest map it is clear that there are large differences between the two NLC spatial layers, this based to a considerable extent on definition and combination of forest, thicket and woodland classes (Department of Environmental affairs 2018), and that they cannot be used to represent forest distribution in the Soutpansberg (Figure 3).

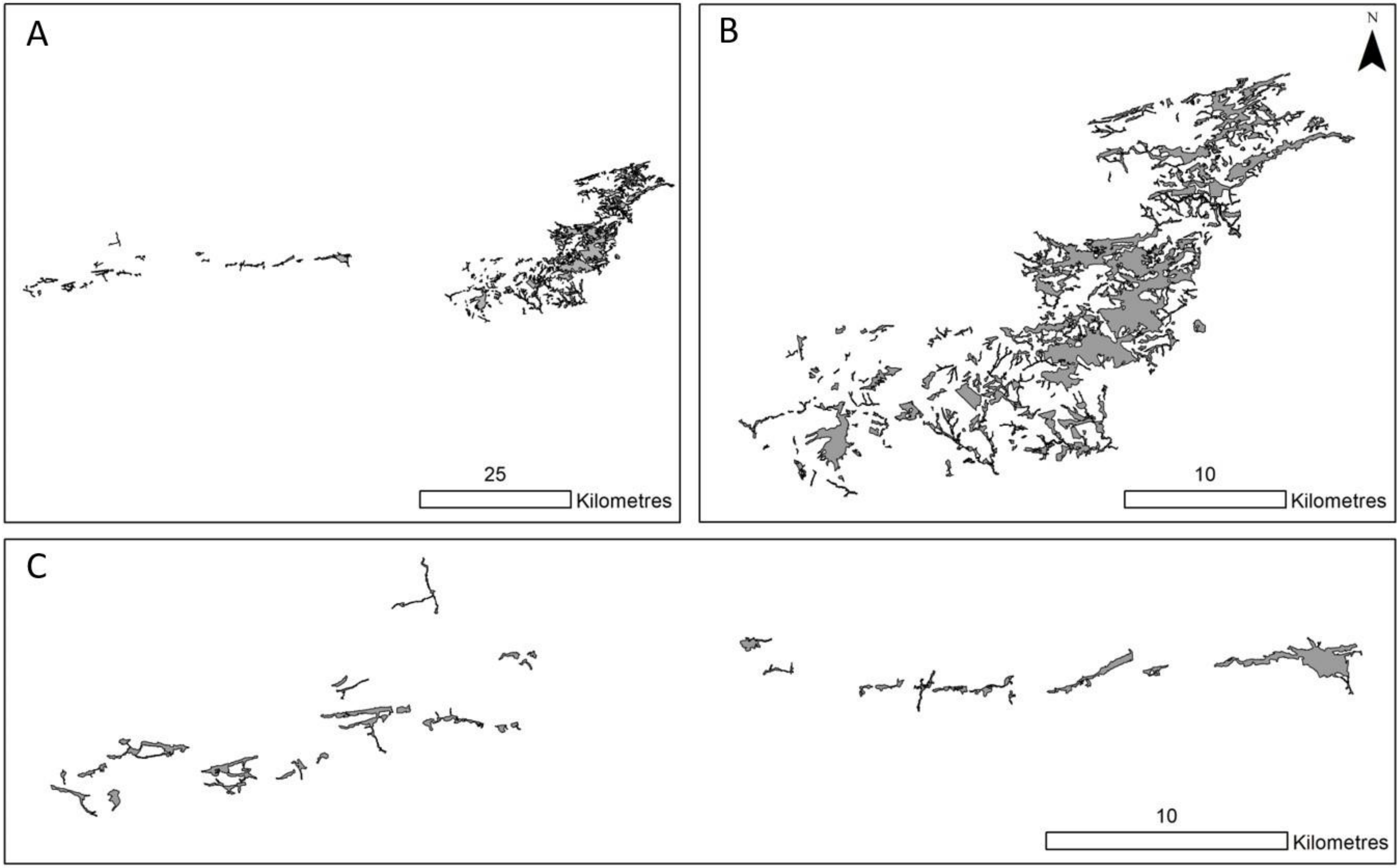

Figure 2. Indigenous forest patches mapped in this study. The Soutpansberg range (A) with larger scale maps of the east (B) and west (C) survey areas.

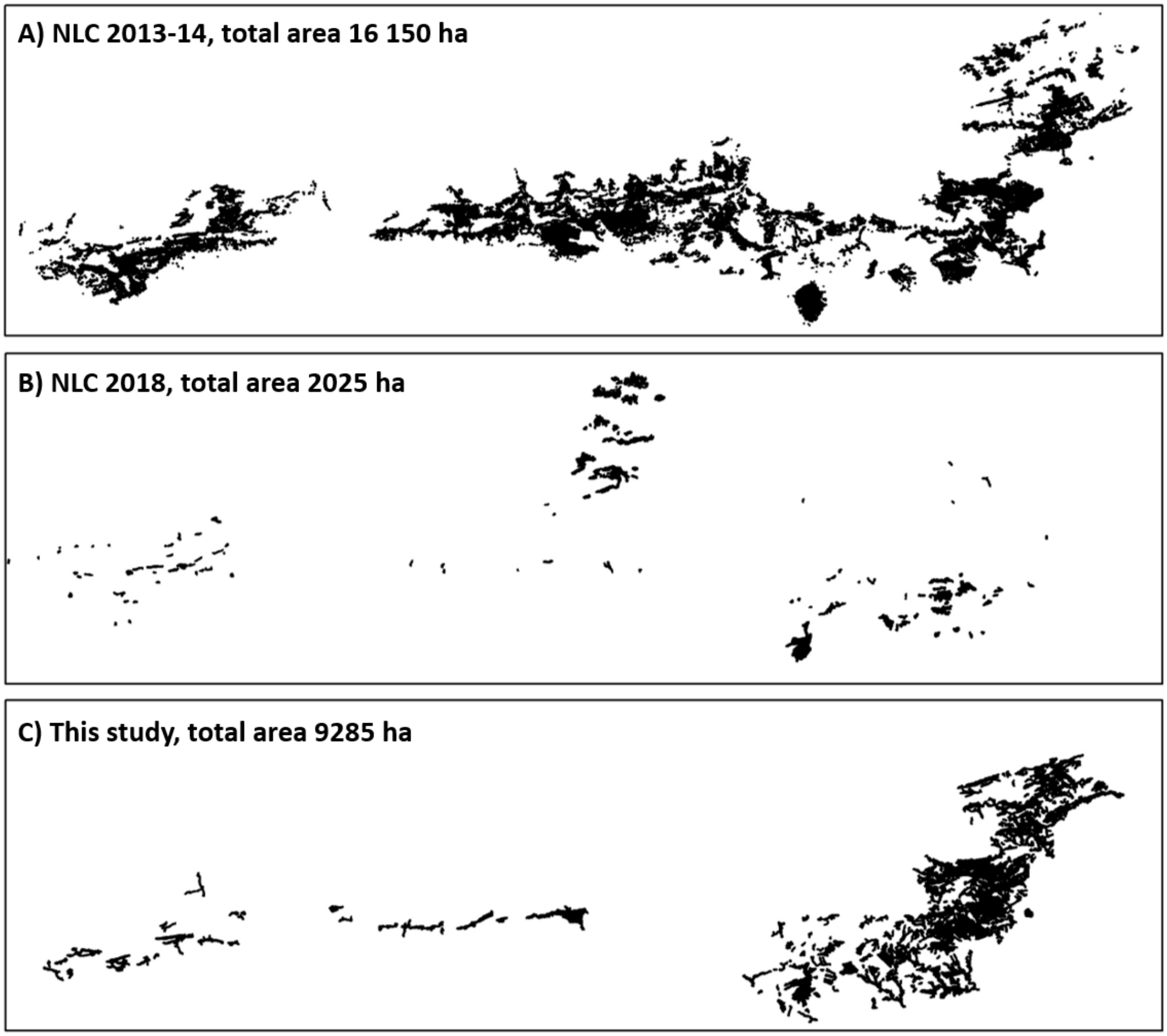

Figure 3. Comparison of spatial layers of forest distribution between the Department of Environmental Affairs national land-cover (NLC) 2013-14 (indigenous forest class) and 2018 (contiguous indigenous forest class) datasets and our study.

3.2. Area of occupancy

Monkey distribution points were 105 km from east to west with a distribution gap of ~ 30 km for in the middle Soutpansberg. We recorded a total of 92 samango monkey distribution points across the Soutpansberg, with 46 records of groups, 43 of lone males or bachelor groups and three ‘unknown’ where a samango monkey was seen but its sex could not be determined (e.g. road crossings) (Table 1). These records are presence data only and do not reflect an accurate overall population size for individual forest patches, as in many cases forest patches likely contain several groups. Of the 233 suitable forest patches identified in the Soutpansberg we surveyed a total of 32 forest patches, 13 in the west and 19 in the east. We aimed to survey at least 50% of the suitable indigenous forest area, resulting in surveying 720 ha (79%) of the 910 ha in the west and 6 583 ha (79%) of the 8 375 ha in the east. We were not able to confirm samango monkey distribution in the Makgabeng plateau despite extensive surveys (six days) and also did not encounter any signs of samango monkeys on the Blouberg inselberg (three days).

Table 1. Samango monkey distribution records collected in the Soutpansberg.

	Total records	Total groups	Total males	Total unknown
West	30	19	10	1
East	62	27	33	2
	92	**46**	**43**	**3**

In the western Soutpansberg, the area of suitable forest habitat was calculated at 910 ha encompassing 31 forest patches (Table S.1) of which all but one was < 100 ha in size and 12 < 10 ha (Figure 4). We confirmed samango monkey occupancy in 12 (39%) of these forest patches, all < 100 ha (range 11 ha – 81 ha) in size (Table 2, Figure 4). This resulted in an area of occupancy (AOO) of 440 ha, representing occupancy in 48% of the suitable forest habitat area. The average SI of occupied forest patches was 4.25 and the average scaled SI was 0.51. The largest forest patch in the west, Hanglip State Forest (281 ha), was found unoccupied. The average size of occupied forest patches was 37 ha. Of the suitable forest habitat 26% (234 ha) is under formal protection and of the forest area occupied 41% (181 ha) are within formally protected areas. Here, 32 % (n = 10) of the 31 suitable forest patches and 42% (n = 5) of the 12 occupied forest patches are partially or fully protected (Table 2). In terms of land ownership 51% of the available forest habitat is under private, 11% under community and 38% under state ownership. Of the samango monkeys' western AOO the majority is under private ownership (Figure 5).

In the eastern Soutpansberg, the suitable forest habitat available was calculated at 8 375 ha encompassing 202 forest patches (Table S.1). Of these 96% (n = 194) were < 100 ha and 74% (n

= 150) < 10 ha in size (Figure 4). Samango monkey occupancy was confirmed in 18 (9%) forest patches ranging in size between 3 ha and 3 762 ha (Table 2, Figure 4). Of the 150 patches < 10 ha three (2%) were found occupied. One 38 ha forest patch surveyed (Lwamondo) was found unoccupied. The AOO was calculated at 6 545 ha, representing occupancy in 78% of the suitable forest habitat. The average SI of occupied forest patches was 5.24 and the average scaled SI was 0.32. Of the suitable forest habitat 15% (1280 ha) falls into formally protected areas and of the forest patches occupied by samango monkeys 17% (1101 ha) are under formal protection. Here, two of the 202 suitable forest patches and one of the 18 occupied forest patches are partially or fully protected (Table 1). Regarding land ownership, 15% of the available forest habitat is under private, 17% under community and 68% under state ownership. Of the samango monkeys' eastern AOO most of it is under state ownership (Figure 5).

Table 2. Forest patches occupied by samango monkeys in the western and eastern Soutpansberg detailing the name (derived from cadastral layers where available), area in hectares (ha), area (ha) under protection, percentage of area under protection and shape index (SI). The scaled SI was calculated separately for the western and eastern Soutpansberg.

	Name	**Area (ha)**	**Area (ha) protected**	**% protected**	**SI**	**Scaled SI**
West						
	Bergplaats	81	69	85	5.85	0.81
	Ottoshoek-Dundee	81	0	0	5.63	0.78
	Hillside-Holworth	69	0	0	4.34	0.6
	Ontmoet-Diepkloof	65	65	100	5.5	0.76
	Buffels Kom	28	0	0	2.72	0.38
	Sarum-Happy Rest	23	15	65	4.24	0.59
	Buysdorp east	21	0	0	1.68	0.23
	Bangor-Carnarvon	18	0	0	7.21	1
	Happy Rest	17	17	100	6.26	0.87
	Ontmoet	15	15	100	2.95	0.41
	Uniondale 1	11	0	0	2.07	0.29
	Ottoshoek	11	0	0	2.49	0.35
East	Entabeni central	3 762	1 101	29	15.88	0.98
	Thathe Vondo central	1 541	0	0	16.28	1
	Luonde	395	0	0	3.73	0.23
	Patch 1	149	0	0	5.81	0.36
	Thathe Vondo east	129	0	0	5.66	0.35
	Patch 2	128	0	0	5.05	0.31
	Patch 3	97	0	0	9.01	0.55
	Shefeera	86	0	0	4.21	0.26
	Patch 4	67	0	0	1.87	0.11
	Patch 5	55	0	0	5.37	0.33
	Vondo dam south	34	0	0	4.5	0.28
	Patch 6	28	0	0	4.39	0.3
	Patch 7	25	0	0	2.49	0.15
	Patch 8	18	0	0	1.39	0.09
	Patch 9	13	0	0	1.69	0.1
	Patch 10	10	0	0	2.4	0.15
	Patch 11	5	0	0	1.75	0.12
	Patch 12	3	0	0	2.79	0.17
TOTAL		**6 985**	**1 282**	**18**	**-**	**-**

Of the 9 285 ha of suitable forest habitat available (233 forest patches) across the Soutpansberg, samango monkeys were found to occupy 75% (6 985 ha) across 30 (13%) forest patches. Of the available forest area 16% (1 514 ha) is under formal protection and of the forest occupied by samango monkeys 18% (1 282 ha) falls within formally protected areas. The majority (n = 224, 96%) of forest patches across the Soutpansberg are < 100 ha in size, with forest patches > 1 000 ha found only in the eastern parts of the mountain range (n = 2) (Figure 4). Samango monkeys occupied forest patches ranging from 3 ha – 3 762 ha.

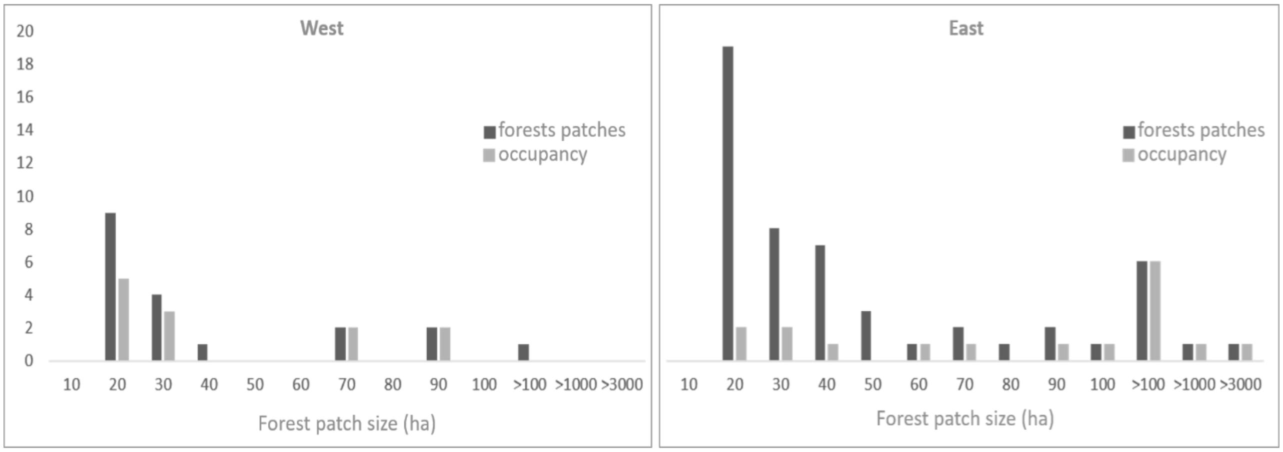

Figure 4. Frequency distributions of different sized forest patches available to, and occupied by, samango monkeys across the western and eastern Soutpansberg. For display, forest patches < 10 ha were excluded from this figure.

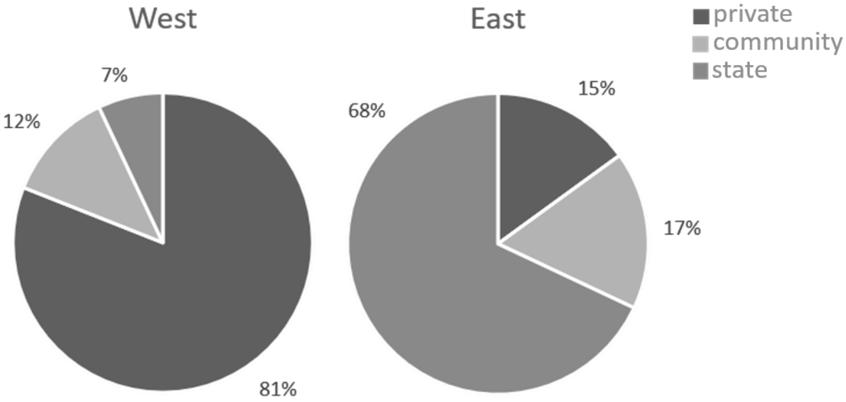

Figure 5. Proportion of the three land ownership categories across the samango monkeys' area of occupancy (AOO) in the western and eastern Soutpansberg.

3.3. Matrix utilisation

Largely through public engagement, we were able to record 62 incidences of samango monkeys in the matrix surrounding forest habitat (Table 3). Here 60% of the records (n = 37) were of lone or bachelor group males and 40% (n = 23) were of groups. Samango monkeys were recorded to utilise timber plantations, fruit orchards (pecan, macadamia, avocado and mango), roads, residential gardens and bushveld. A third of all records were either roadkills or road crossings (n = 23, 37%) in the eastern Soutpansberg which affected males and groups equally (Table 3) showing that paved roads pose no barrier to samango monkeys. Records from samango monkeys in residential gardens showed that males utilised them more frequently (n = 16, 89 %) than females (n = 2, 11%).

Table 3. Number of samango monkey distribution points recorded in the matrix and in the different matrix components, divided into males (lone or bachelor), groups and 'unknown' compared between the eastern and western Soutpansberg.

		Matrix	Plantation	Orchards	Road	Garden	Bushveld
Males	**East**	27	1	3	12	11	-
	West	10	-	-	-	5	5
Groups	**East**	18	-	6	9	2	1
	West	5	-	-	-	-	5
Unknown	**East**	2	-	-	2	-	-
	West	-	-	-	-	-	-
TOTAL	**East**	**47**	**1**	**9**	**23**	**13**	**1**
	West	**15**	**-**	**-**	**-**	**5**	**10**

Roadkills and crossings records showed that timber plantations (*Eucalyptus* and *Pinus* spp.) leading up to roads were utilised in 35% (n = 8) of the recorded cases (n = 23). Although these timber plantations seemingly play a role in connecting suitable forest patches, rotational clear-felling of these can result in regular loss of potential corridors (Figure 6).

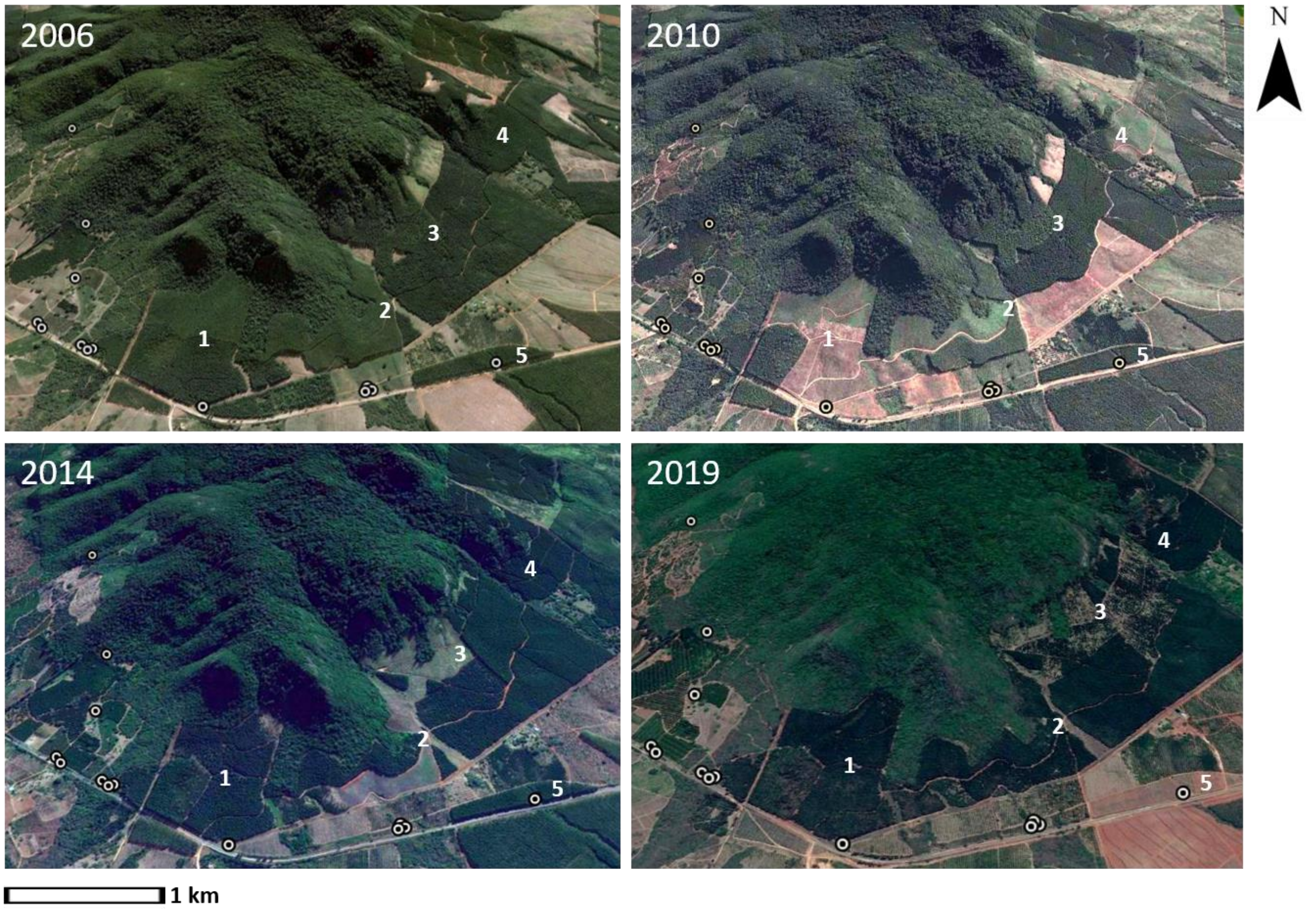

Figure 6. Landscape change through regular felling of timber plantations and loss of possible samango monkey corridors on the edges of the Luonde forest between 2006, 2010, 2014 and 2019. Points indicate samango monkey distribution records between 2012 - 2017 and numbers 1-5 highlight different areas affected by change. Map data: Google, Maxar Technologies.

Another measurement we used for matrix utilisation is the maximum distances samango monkeys were found away from the nearest suitable forest patch when in the matrix (Table 4). As samango monkeys are male dispersed we also calculated the maximum distance males were found away from the next group occupying a forest patch. The maximum distance from the nearest forest patch was 1.3 km for groups and 2.4 km for males and the maximum distance of males from the next group was 4.1 km.

Table 4. Maximum distances (km) of lone or bachelor group males and groups recorded from the nearest suitable forest patch and of males to the nearest group occupying a forest patch in the eastern and western Soutpansberg.

		distance nearest forest	**distance nearest group**
Groups	**East**	0.6	-
	West	1.3	-
Males	**East**	1.4	2.4
	West	2.4	4.1

3.4. Forest connectivity

We used the maximum distances to the nearest forest patch found for groups to infer the possibility of groups colonising unoccupied patches or re-occupying patches after a local extinction event and we used the maximum distances to the nearest groups occupying a forest patch found for males to show the likelihood of genetic connectivity through males moving between occupied patches (Table 4). This was done by buffering each forest patch by those maximum distances for groups and males to create a landscape of connectivity by using distance only (Figure 7 and 8).

Looking at the possibility of groups re-colonising forest patches in an event of local extinction in the west it can be seen that three forest patches are completely isolated by distance while all the others could in theory be reached by groups (Figure 7A). There is also a clear separation between forests in the far west and all the others in the west (Figure 7A). When looking at unoccupied forest patches in the west it can be seen that all unoccupied forest patches are at distances to occupied forest patches that could be overcome by groups and the unoccupied forest patches could act as “stepping stones” between occupied forest patches (Figure 7A). For the eastern Soutpansberg it is shown that there are two main areas of occupancy which are isolated from each other. Again, unoccupied forest patches would be in reach and could act as stepping stones between those two areas given a local extinction event (Figure 7B). The pattern in the east suggests a mainland-island situation with the large forest patches likely being source populations to smaller forest patches surrounding them. Unoccupied forest patches are theoretically all within reach of groups (Figure 7B).

When looking at the genetic connectivity facilitated by male dispersal, western populations are again separated into two (Figure 8). In the east, males could theoretically facilitate genetic connectivity between all occupied forest patches.

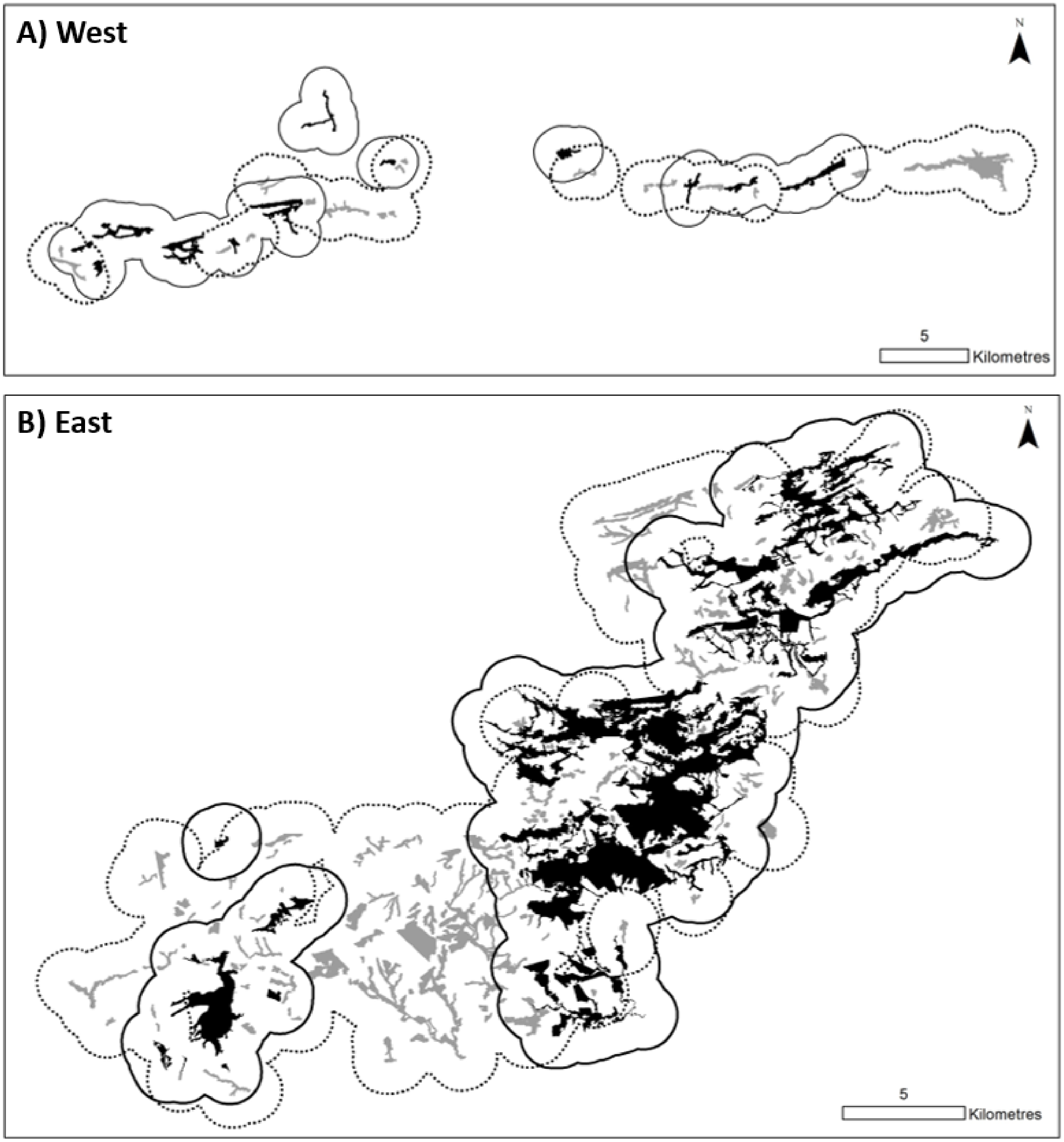

Figure 7. Inferring possibility of maternal groups colonising unoccupied forest fragments or re-occupying forest fragments after a local extinction event in the west (A) and the east (B) using the maximum distance of 1.3 km groups have been found away from the nearest forest patch. Black

indicates occupied forest patches and light grey unoccupied. Solid lines show the 1.3 km buffer around occupied forest patches and dashed lines the 1.3 km buffer around unoccupied forest patches.

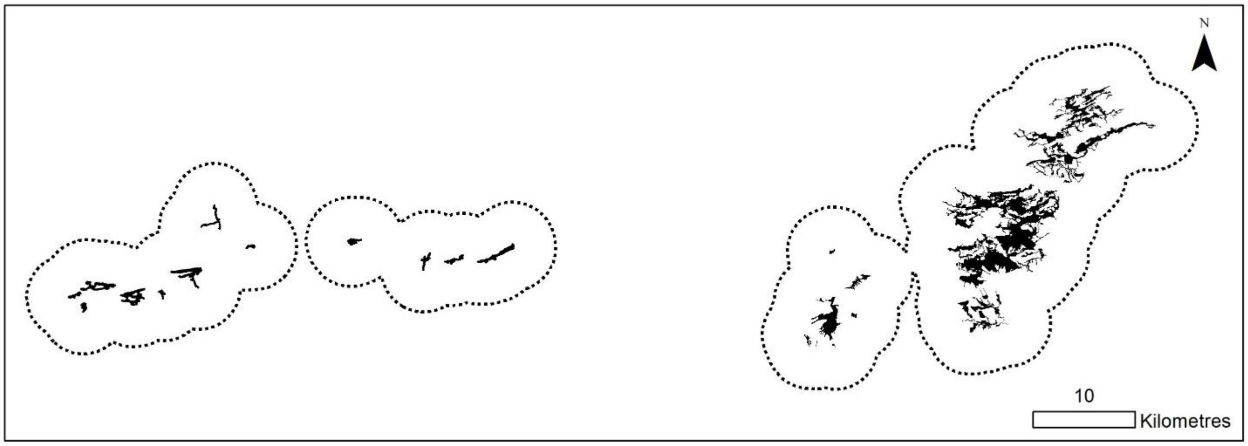

Figure 8. Inferring genetic connectivity through males between occupied forest patches, using the maximum distance of 4.1 km males were found away from the nearest group in a forest patch, across all forest patches in the Soutpansberg. Black indicates occupied forest patches and dashed lines indicate 4.1 km buffers around group-occupied forest patches.

4. Discussion

4.1. Samango monkey occupancy

Samango monkeys were found to occupy 75% of the available forest area across 30 forest patches in the Soutpansberg. Comparing the eastern and western Soutpansberg, we found that the east contains a larger total area of forest cover (east 8 375 ha, west 910 ha) and more (east 202, west 31) and larger forest patches (maximum size east 8 375 ha, maximum size west 281 ha). The average scaled SI for forests in the east was lower (0.32) than for forests in the west (0.51).

The Soutpansberg samango monkeys were found largely in forest patches < 100 ha in size and our results show that overall, the Soutpansberg has very few forest patches > 100 ha available (n = 9, 4%). This is in contrast to what Lawes (1992) found for patch occupancy in KwaZulu Natal where only patches > 100 ha were occupied, and where the likelihood of occupancy decreased as forest patches become smaller.

In the east, 74% of forest patches are < 10 ha which indicates a high degree of fragmentation in that landscape. We recorded samango monkeys occupying patches < 10 ha three times in the east and ~ 10 ha twice in the west and a possible explanation for this could be that they are using several of these smaller patches, moving between them through the surrounding matrix. Especially the bushveld surrounding forests in the west not only offers connectivity but also suitable food resources such as certain tree and creeper species (e.g. *Vachellia* (*Acacia*) *karroo*, *Senegalia* (*Acacia*) *ataxacantha*, *Olea europaea*, *Capparis fascicularis*, *Dovyalis zeyheri*; Linden et al. 2015) allowing samango monkeys to extend their home ranges into this woodland habitat type. Lawes (1992) found for samango monkeys in coastal dune and scarp forests that adjacent thicket/woodland constituted about 14% of their home ranges and was used intensively for resting, moving, and social behaviour. The species has generally been suggested to show a comparatively broad forest habitat tolerance compared to other forest guenons (Lawes 1990). An interesting question in this context would be: have populations increased in the past century with the increase of woody vegetation cover and loss of grasslands (also recorded for the survey area; Hahn 2017a) surrounding forests?

Our results show that that there is a ~ 30 km distribution gap in the middle Soutpansberg separating samango monkey populations in the east from populations in the west. Within this gap lies the largest forest patch (Hanglip forest) of the western Soutpansberg which is however unoccupied by samango monkeys. The absence of samango monkeys in this large forest patch was previously noted by von dem Bussche & van der Zee (1985). We consider the forest patch as highly suitable for samango monkeys in terms of structure and size and suggest that the reason for their absence is likely due to a local extinction event and a lack of connectivity hampering subsequent re-colonisation. Causes for a local extinction are not known but it could be due to either natural events (e.g. extinction vortex though mutual reinforcement of abiotic and biotic factors such as environmental stochasticity, demographic stochasticity, inbreeding, and behavioural failures, driving population size downward to extinction (Gilpin & Soulé 1986)), direct anthropogenic threats (hunting) or a combination of the two.

We suspect a similar situation for the forests of the Blouberg inselberg and for the riverine forest of the Makgabeng cuesta where we did not encounter any samango monkeys.

4.2. *Matrix utilisation and forest connectivity*

Our results show that samango monkeys used the surrounding matrix in both the west where the matrix is largely comprised of bushveld and in the east where the matrix is dominated by human settlements, infrastructure and commercial agricultural and silvicultural land. It is noteworthy that lone or bachelor groups males were regularly recorded in residential gardens and it was reported by respondents to the public calls that these males would sometimes stay for several months. Residential gardens in the study area are generally characterised by the presence of several large trees (often including *Ficus* spp.) which could be a valuable resource offering both shelter and food for these males (Asensio et al. 2009).

Groups and lone or bachelor groups males were observed in fruit orchards reportedly feeding on unripe macadamia nuts. For groups this only occurred in orchards directly bordering indigenous forest whereas males regularly associated with groups of vervet monkeys when encountered on foraging sorties in orchards (Linden B. pers. obs.). Given this utilisation of orchards, we suggest that these can also play a potential role as corridors at least for lone or groups of bachelor males moving through the matrix. Our large number of records of road fatalities and road crossings indicate that roads do not pose a barrier (although they are a danger) to samango monkeys when moving through the matrix. Similar observations were made for timber plantations. Several roadkill/crossing localities indicated that these plantations were seemingly utilised as corridors. However, given the regular harvesting cycles (25-30 years for *Pinus* spp. trees, 7-30 years for *Eucalyptus* spp. trees depending if they are used for pulp and mining timber or for furniture; Bredenkamp & Upfold 2012) of timber plantation stands through clear-felling, these potential corridors can disappear abruptly resulting in loss of connectivity (Figure 6). We further found from the samango monkey distribution data that riverine forests particularly in the east play an important role as natural corridors.

Forest patch (re)colonisation and dispersal between patches not only depends on the presence of corridors and the land use patterns in the matrix (Arroyo-Rodríguez & Mandujano 2009; Estrada & Coates-Estrada 1996) but will also depend on the distance between patches in relation to distances that samango monkeys are comfortable to move away from primary forest habitat. Here we found that the maximum distance for males was 4.1 km and for groups 1.3 km.

Female samango monkeys are highly philopatric and we suggest that forest patch (re)colonisation most likely occurs through group fission (due to increasing group size) into sister-groups with subsequent range extension/shifts (Swart & Lawes 1996; Linden pers. obs.). Our results show that groups might not be able to recolonise some of the forest patches in the eastern and western Soutpansberg in cases of local extinctions unless they make use of unoccupied forest patches as stepping stones to bridge gaps. Of the unoccupied forest patches, groups could theoretically occupy all of them, however it is not certain that the large number of patches < 10 ha found in the east could on their own permanently sustain groups of samango monkeys. Male samango monkeys moved much greater distances away from forests then females. As males must leave their maternal group after reaching sexual maturity, they might sometimes be forced to range far, taking large risks to find a new group of females to reproduce with (Swart & Lawes 1996). In terms of distance between occupied forest patches, males could theoretically facilitate genetic connectivity between most of them.

We found three distance-based barriers across the Soutpansberg. One between the furthest east and the next occupied neighbouring forest patch to the west, one separating all forests in the east from the west and one between the furthest western forests and the neighbouring forests to the east (Figure 3, 7 and 8). The latter distance gap found in the western Soutpansberg also correlates with an arid low-lying geographic barrier, the Sand River gorge, in and around which we found no suitable forest patches. The major distance gap found in the east, that separating the Entabeni/Thathe Vondo forests from the Luonde forest, comprises of a heavily transformed agricultural and silvicultural landscape which does however have small suitable forest patches available to bridge the gap. The widest gap (~ 30 km) separates eastern and western populations entirely, leaving the west without any possibilities of a rescue effect if populations should decline or become locally extinct.

4.3. Threats

Our study showed that less than a quarter of the suitable forest habitat available for samango monkeys and again less than a quarter of their AOO are under formal protection. In the east both the available and the occupied forest habitat receive < 20% formal protection although a portion of the Thathe Vondo forests fall within the buffer zone of the Lake Fundudzi cultural landscape

National Heritage Site (Republic of South Africa 2014). The situation is slightly better in the west with both available and occupied forest habitat receiving ~ 30% formal protection. Nationally, all indigenous forests are protected under the National Forests Act [Act No 84 of 1998]; however, due to considerable legal and illegal exploitation the conservation status of much indigenous forest is uncertain (Castley & Kerley 1996; Lawes et al. 2001). The fact that forest areas in the Soutpansberg are variously private, community or state owned further complicates consistent forest conservation and management and environmental law enforcement. In the west the majority of forests occupied by samango monkeys are under private landownership and we consider changes in private landowners a potential risk to forest and samango monkey protection.

In the east, ongoing deforestation for small scale farming (Munyati & Kabanda 2008) and commercial agriculture (Griscom et al. 2010; Linden B. pers. obs.) is considerable in certain areas, posing a threat to the samango monkeys' primary habitat. Since the majority of forest area is state owned in the eastern Soutpansberg, indigenous forest protection and management could be improved through stricter law enforcement.

The considerable degree of matrix utilisation found for lone and bachelor groups males and to a lesser extent maternal groups in the human dominated landscape of the eastern Soutpansberg exposes the samango monkeys to a variety of threats, particularly road fatalities, which could hamper male dispersal between groups and lead to overall population reduction locally.

Global climate change is increasingly regarded an important threat to biodiversity which needs to be considered for conservation strategies to be successful (Walther et al. 2002). Current research contends that the most significant threats are drying trends, changes in rainfall patterns, changes to fire regimes and changes in seasonality which in turn lead to changes in species distribution and composition (Hannah et al. 2002; Midgley et al. 2002; Berliner 2005). Predictions for the north-eastern parts of South Africa (including the Soutpansberg) show reduced annual average rainfall while average annual temperatures will continue to rise (Thomas et al. 2007; Kruger & Sekele 2013; Department of Environmental Affairs 2013; Engelbrecht et al. 2016). This predicted drying trend might result in forest tree die-offs, plant community changes, overall forest cover shrinkage (Rutherford et al. 1999) and increased susceptibility to disturbances. Given the forecast rapidity of climate change combined with the anthropogenic constraints on the landscape, forest and samango monkeys could be impacted substantially in future.

5. Conclusion

Our study shows that samango monkeys in the Soutpansberg occupy a large range of forest patch sizes, utilise all components of the matrix surrounding forests and that males and females differ in matrix utilisation, with males using the matrix more extensively considering maximum distances, time spent in the matrix and components of the matrix used. We could show that the regular matrix utilisation exposes samango monkeys to specific threats linked to human infrastructure and activities. Patterns found in this study differ between the contrasting landscapes the samango monkeys are associated with in the west and east, requiring different adaptive strategies from the monkeys and different conservation and management approaches from practitioners. While the main focus in the west should lie with the continued protection of remaining forest habitat and protected area expansion, the picture in the east is more complex. Here adequate corridor management should be established, small forest fragments within the matrix be actively protected as stepping stones, road fatalities be mitigated, total area of forest under formal protection be increased and better law enforcement for the protection of forests implemented. Overall, we consider samango monkey populations in the east to be more vulnerable despite more and larger forest areas available and a lower average SI of forest patches compared to populations in the west. Our results support previous studies (Lawes 1990; Lawes 1992; Lawes 2000) that noted the tolerance of samango monkeys to matrix modifications having evolved in repeatedly fragmented forest habitat (Botzat et al. 2015) since radiating out of East Africa into southern Africa in the mid-Pleistocene (Dalton et al. 2015).

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Supplementary

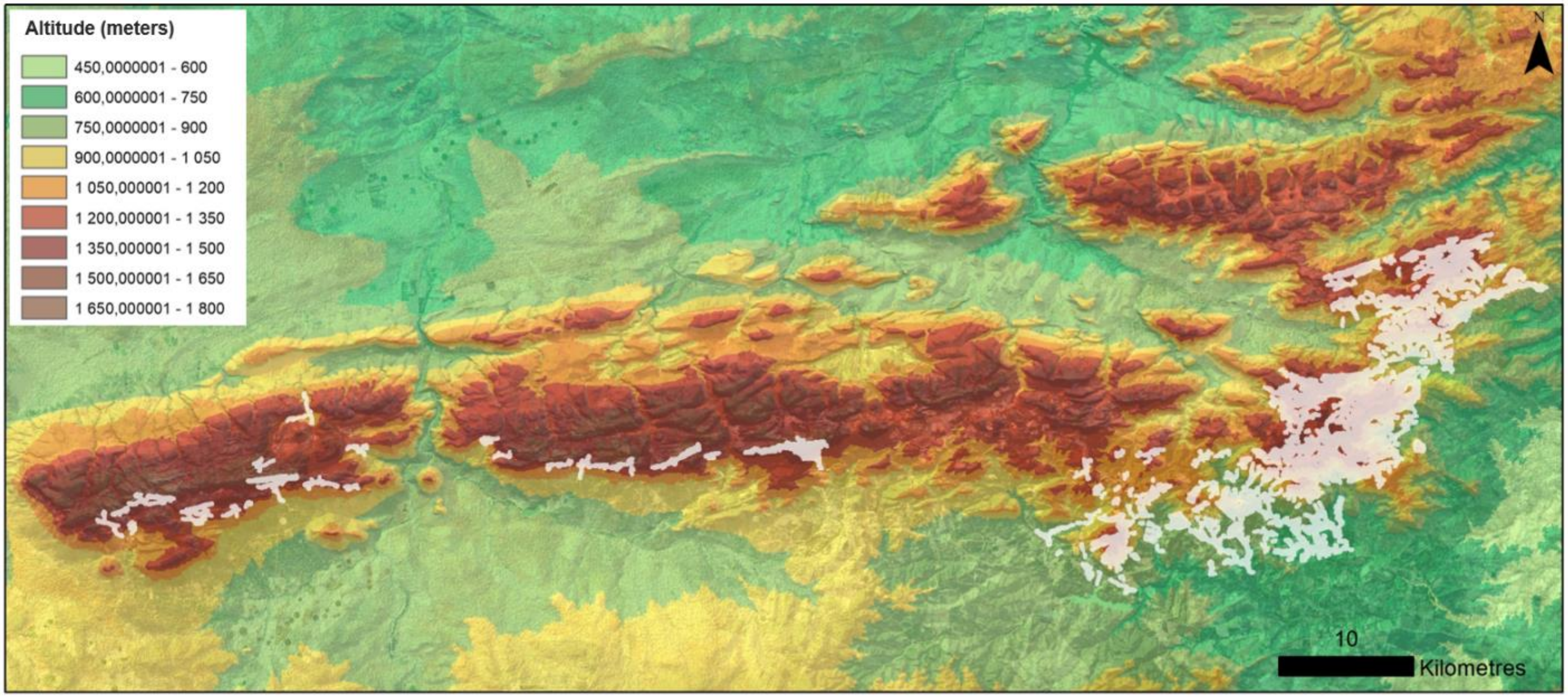

Figure S.1. Relief map (NASA Shuttle Radar Topography Mission, 1 arc-second) and distribution of forests (opaque white) across the study area illustrating the altitudinal range across which they are found.

Table S.1. List of all forest patches mapped across the Soutpansberg including the survey area, patch ID, occupancy by samango monkeys, area in ha and m2, perimeter in m, shape index (SI) and scaled shape index.

Survey area	Id	Occupancy	Area (ha)	Area (m2)	Perimeter (m)	SI	SI scaled
West	Uniondale 1	1	11	108892	2423	2.07	0.29
West	Sarum-Happy Rest	1	23	234178	7267	4.24	0.59
West	Ottoshoek-Dundee	1	81	807261	17934	5.63	0.78
West	Ottoshoek	1	11	108366	2908	2.49	0.35
West	Ontmoet-Diepkloof	1	65	645694	15660	5.50	0.76
West	Ontmoet	1	15	146771	4002	2.95	0.41
West	Hillside-Holworth	1	69	688757	12766	4.34	0.60
West	Happy Rest	1	17	168544	9109	6.26	0.87
West	Buysdorp east	1	21	212768	2741	1.68	0.23
West	Buffels Kom	1	28	280768	5116	2.72	0.38
West	Bergplaats	1	81	811774	18683	5.85	0.81
West	Bangor-Carnarvon	1	18	179023	10819	7.21	1.00
West	173	0	12	117220	1761	1.45	0.20
West	158	0	7	70571	1585	1.68	0.23
West	131	0	7	74135	1839	1.91	0.26
West	122	0	5	45849	1474	1.94	0.27
West	119	0	8	77953	1944	1.96	0.27
West	113	0	7	73512	1942	2.02	0.28
West	107	0	5	46410	1619	2.12	0.29
West	96	0	5	51340	1783	2.22	0.31
West	79	0	4	43653	1892	2.55	0.35
West	77	0	13	134350	3324	2.56	0.35
West	73	0	7	71934	2455	2.58	0.36
West	54	0	15	151525	3899	2.83	0.39
West	49	0	4	40648	2125	2.97	0.41
West	29	0	24	240498	6402	3.68	0.51
West	28	0	17	167917	5425	3.73	0.52
West	21	0	9	91462	4372	4.08	0.57
West	14	0	7	71930	4306	4.53	0.63
West	Hanglip	0	281	2809010	27847	4.69	0.65
West	10	0	33	329581	9838	4.83	0.67
East	Vondo dam south	1	34	339808	9297	4.50	0.28
East	Thathe Vondo east	1	129	1290990	22784	5.66	0.35
East	Thathe Vondo central	1	1541	15414700	226543	16.28	1.00
East	Shefeera	1	86	859090	13849	4.21	0.26
East	Patch 9	1	13	131247	2168	1.69	0.10
East	Patch 8	1	18	179954	2088	1.39	0.09
East	Patch 7	1	25	254751	4455	2.49	0.15
East	Patch 6	1	28	275500	8164	4.39	0.27

East	Patch 5	1	55	547784	14084	5.37	0.33
East	Patch 4	1	67	673750	5441	1.87	0.11
East	Patch 3	1	97	970617	31453	9.01	0.55
East	Patch 2	1	128	1278450	20238	5.05	0.31
East	Patch 12	1	3	30591	1732	2.79	0.17
East	Patch 11	1	5	45659	1325	1.75	0.11
East	Patch 10	1	10	97089	2652	2.40	0.15
East	Patch 1	1	149	1487950	25108	5.81	0.36
East	Luonde	1	395	3953280	26291	3.73	0.23
East	Entabeni central	1	3762	37620900	345172	15.88	0.98
East	203	0	2	24115	607	1.10	0.07
East	202	0	2	15823	506	1.14	0.07
East	201	0	1	11098	425	1.14	0.07
East	200	0	2	24210	634	1.15	0.07
East	199	0	1	13928	481	1.15	0.07
East	198	0	2	19216	571	1.16	0.07
East	197	0	3	32705	759	1.18	0.07
East	196	0	1	14915	522	1.21	0.07
East	195	0	1	10496	440	1.21	0.07
East	194	0	2	20908	624	1.22	0.07
East	193	0	3	30075	762	1.24	0.08
East	192	0	3	26681	722	1.25	0.08
East	191	0	1	14919	540	1.25	0.08
East	190	0	2	21590	651	1.25	0.08
East	189	0	3	28908	753	1.25	0.08
East	188	0	2	22302	669	1.26	0.08
East	187	0	4	39947	907	1.28	0.08
East	186	0	1	13663	540	1.30	0.08
East	185	0	2	18144	627	1.31	0.08
East	184	0	5	48771	1043	1.33	0.08
East	183	0	1	12814	536	1.34	0.08
East	182	0	4	36871	918	1.35	0.08
East	181	0	2	20594	686	1.35	0.08
East	180	0	11	111962	1601	1.35	0.08
East	179	0	2	23910	740	1.35	0.08
East	178	0	119	1193590	5282	1.36	0.08
East	177	0	1	13311	560	1.37	0.08
East	176	0	1	11365	525	1.39	0.09
East	175	0	2	24336	777	1.40	0.09
East	174	0	2	22733	761	1.42	0.09
East	172	0	2	23437	794	1.46	0.09
East	171	0	2	23255	792	1.46	0.09
East	170	0	1	10153	536	1.50	0.09
East	169	0	2	22608	806	1.51	0.09
East	168	0	6	55954	1277	1.52	0.09
East	167	0	1	10203	559	1.56	0.10

East	166	0	9	94757	1713	1.57	0.10
East	165	0	2	24869	884	1.58	0.10
East	164	0	1	11916	613	1.59	0.10
East	163	0	2	16142	729	1.62	0.10
East	162	0	3	34193	1063	1.62	0.10
East	161	0	1	13722	687	1.66	0.10
East	160	0	2	24293	921	1.67	0.10
East	159	0	1	10536	610	1.68	0.10
East	157	0	4	38152	1168	1.69	0.10
East	156	0	4	44222	1260	1.69	0.10
East	155	0	4	42409	1241	1.70	0.10
East	154	0	38	378717	3764	1.73	0.11
East	153	0	2	23691	943	1.73	0.11
East	152	0	3	25327	986	1.75	0.11
East	151	0	1	10744	646	1.76	0.11
East	150	0	2	18995	860	1.76	0.11
East	149	0	2	21989	927	1.76	0.11
East	148	0	4	38771	1236	1.77	0.11
East	147	0	5	52468	1442	1.78	0.11
East	146	0	3	34428	1170	1.78	0.11
East	145	0	2	22262	946	1.79	0.11
East	144	0	4	36766	1236	1.82	0.11
East	143	0	2	19117	892	1.82	0.11
East	142	0	7	72934	1750	1.83	0.11
East	141	0	4	39503	1289	1.83	0.11
East	140	0	9	85648	1899	1.83	0.11
East	139	0	6	62522	1624	1.83	0.11
East	138	0	2	17426	859	1.84	0.11
East	137	0	3	27237	1082	1.85	0.11
East	136	0	1	11799	718	1.86	0.11
East	135	0	1	11998	734	1.89	0.12
East	134	0	4	35315	1261	1.89	0.12
East	133	0	4	35996	1279	1.90	0.12
East	132	0	2	18012	906	1.91	0.12
East	130	0	2	15397	843	1.92	0.12
East	129	0	1	10007	681	1.92	0.12
East	128	0	8	79691	1921	1.92	0.12
East	127	0	8	83887	1972	1.92	0.12
East	126	0	4	35891	1294	1.93	0.12
East	125	0	1	14311	818	1.93	0.12
East	124	0	2	23328	1045	1.93	0.12
East	123	0	6	56103	1625	1.93	0.12
East	121	0	3	29333	1184	1.95	0.12
East	120	0	6	63472	1749	1.96	0.12
East	118	0	2	21096	1017	1.98	0.12
East	117	0	2	24006	1089	1.98	0.12

East	116	0	1	13161	808	1.99	0.12
East	115	0	4	37501	1365	1.99	0.12
East	114	0	37	366201	4267	1.99	0.12
East	112	0	4	37375	1386	2.02	0.12
East	111	0	17	165462	2931	2.03	0.12
East	110	0	2	16795	960	2.09	0.13
East	109	0	7	72602	1995	2.09	0.13
East	108	0	8	78422	2102	2.12	0.13
East	106	0	4	35756	1422	2.12	0.13
East	105	0	2	19971	1071	2.14	0.13
East	104	0	2	19238	1060	2.16	0.13
East	103	0	2	15917	966	2.16	0.13
East	102	0	17	168937	3173	2.18	0.13
East	101	0	2	15007	949	2.19	0.13
East	100	0	2	21116	1126	2.19	0.13
East	99	0	4	38140	1518	2.19	0.13
East	98	0	8	75963	2154	2.20	0.14
East	97	0	20	196082	3479	2.22	0.14
East	95	0	7	74354	2193	2.27	0.14
East	94	0	2	21209	1173	2.27	0.14
East	93	0	6	60503	1999	2.29	0.14
East	92	0	2	19839	1155	2.31	0.14
East	91	0	3	30807	1442	2.32	0.14
East	90	0	2	16381	1064	2.35	0.14
East	89	0	4	39620	1661	2.35	0.14
East	88	0	2	19520	1186	2.40	0.15
East	87	0	3	25854	1367	2.40	0.15
East	86	0	6	63579	2181	2.44	0.15
East	85	0	2	19786	1226	2.46	0.15
East	84	0	15	151271	3436	2.49	0.15
East	83	0	4	36305	1688	2.50	0.15
East	82	0	3	33968	1639	2.51	0.15
East	81	0	3	27833	1489	2.52	0.15
East	80	0	1	12527	1006	2.54	0.16
East	78	0	4	43941	1901	2.56	0.16
East	76	0	4	40082	1817	2.56	0.16
East	75	0	1	13142	1041	2.56	0.16
East	74	0	5	53441	2102	2.57	0.16
East	72	0	7	66949	2376	2.59	0.16
East	71	0	16	162000	3721	2.61	0.16
East	70	0	5	51566	2104	2.61	0.16
East	69	0	12	122699	3247	2.62	0.16
East	68	0	3	29451	1601	2.63	0.16
East	67	0	4	36848	1799	2.64	0.16
East	66	0	1	11924	1031	2.66	0.16
East	65	0	5	45494	2020	2.67	0.16

East	64	0	2	19058	1308	2.67	0.16
East	63	0	9	90606	2868	2.69	0.17
East	62	0	7	67919	2491	2.70	0.17
East	61	0	25	246985	4758	2.70	0.17
East	60	0	2	24375	1499	2.71	0.17
East	59	0	1	14698	1179	2.74	0.17
East	58	0	20	202507	4382	2.75	0.17
East	57	0	11	111696	3259	2.75	0.17
East	56	0	8	83208	2852	2.79	0.17
East	55	0	3	26485	1618	2.80	0.17
East	53	0	2	16689	1300	2.84	0.17
East	52	0	14	144675	3858	2.86	0.18
East	51	0	2	17688	1368	2.90	0.18
East	50	0	5	52741	2372	2.91	0.18
East	48	0	19	194706	4661	2.98	0.18
East	47	0	2	15221	1308	2.99	0.18
East	46	0	2	20597	1537	3.02	0.19
East	45	0	5	49007	2384	3.04	0.19
East	44	0	11	106131	3531	3.06	0.19
East	43	0	2	23946	1679	3.06	0.19
East	42	0	8	84291	3157	3.07	0.19
East	41	0	3	26281	1798	3.13	0.19
East	40	0	16	159926	4494	3.17	0.19
East	39	0	6	59167	2777	3.22	0.20
East	38	0	3	27471	1922	3.27	0.20
East	37	0	90	897891	11005	3.28	0.20
East	36	0	5	54334	2760	3.34	0.21
East	35	0	26	256975	6011	3.34	0.21
East	34	0	13	132306	4554	3.53	0.22
East	33	0	2	21855	1856	3.54	0.22
East	32	0	23	232286	6087	3.56	0.22
East	31	0	8	75304	3499	3.60	0.22
East	30	0	7	67275	3368	3.66	0.23
East	27	0	2	16722	1769	3.86	0.24
East	26	0	5	53683	3173	3.86	0.24
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East	24	0	4	38709	2722	3.90	0.24
East	23	0	6	64962	3591	3.97	0.24
East	22	0	6	62556	3561	4.02	0.25
East	20	0	4	44915	3146	4.19	0.26
East	19	0	47	474800	10307	4.22	0.26
East	18	0	35	352475	9007	4.28	0.26
East	17	0	29	288333	8322	4.37	0.27
East	16	0	70	699155	13054	4.40	0.27
East	15	0	8	76570	4360	4.44	0.27
East	13	0	79	789116	14375	4.56	0.28

East	11	0	48	484326	11904	4.83	0.30
East	9	0	29	291579	9414	4.92	0.30
East	8	0	35	352094	10850	5.16	0.32
East	7	0	14	142726	6997	5.22	0.32
East	6	0	13	125824	6703	5.33	0.33
East	5	0	25	250516	9510	5.36	0.33
East	4	0	32	324601	10988	5.44	0.33
East	3	0	31	313718	12734	6.41	0.39
East	2	0	46	461292	16215	6.73	0.41
East	1	0	215	2147560	48893	9.41	0.58

Chapter 3: Potential drivers of samango monkey (*Cercopithecus albogularis schwarzi*) population subdivision in a highly fragmented mountain landscape in northern South Africa

Abstract

Forests affected by fragmentation are at risk of losing their primate populations over the long term. And, although the impact of fragmentation on primate populations has been studied in many places in Africa, Asia and South America there is no discernible pattern of how primates react to forest disturbance and fragmentation. Isolation due to habitat fragmentation has profound effects on the genetic viability of populations as the risk of heterozygosity loss from inbreeding and genetic drift increases. Here we used microsatellite and mitochondrial DNA data to investigate how habitat fragmentation impacts on the genetic diversity and structure of a samango monkey population inhabiting forest patches in a mountain range of northern South Africa. Our analyses show that the studied population is subdivided across the mountain and that the lack of contemporary gene flow likely completely isolates this population from the closest source population further south. Based on our results we suggest that natural and anthropogenic fragmentation and geographical distance are potential drivers for the observed population genetic differentiation and that the matrix of surrounding forests and its suitability for samango utilisation plays a role at the local scale. The degree of samango monkey population subdivision and the apparent lack of contemporary migration between populations found here raises concerns about the long-term viability of populations across the mountain range.

1. Introduction

Forest habitat loss and fragmentation and the resulting isolation of animal populations is a global issue of our time (FAO 2018). African forests are characterised by a diverse mammalian fauna, including primates, which have been shown to play vital roles in forest ecosystem functioning, structure and resilience (Estrada et al. 2017). Primates disperse seeds, play integral roles in food webs as consumers and prey and participate in a diverse array of coevolved relationships with other species (Marsh 2003; Seufert et al. 2009; Linden et al. 2015). Forests affected by outside disturbance and/or isolation are at risk of losing their primate populations over time. Although the impact of fragmentation on primate populations has been studied in many places in Africa, Asia and South America there is no discernible pattern of how primates react to forest disturbance and fragmentation (Marsh 2003; Marsh 2013). This is because the ability of primate populations to sustain themselves in disturbed and fragmented forests is very species- and circumstance-specific and as a result, so are conservation and management recommendations (Gibbons & Hartcourt 2009).

If habitat fragmentation leads to reduced gene flow between populations, it could have profound effects on the genetic viability of populations due to two main genetic problems (Lacy 1997; Dudash & Fenster 2000). Firstly, isolated populations experience greater inbreeding as immigration and emigration are impeded. Inbreeding, especially over several generations, increases the chance of homozygosity of deleterious recessive alleles and thus their expression. At the same time heterozygosity is lost and this can lead to reduced adaptability (Lacy 2000). Secondly, small populations are also more vulnerable to genetic drift causing random changes in allele frequencies, again resulting in a loss of genetic variability (Lacy 1997). The combination of drift and inbreeding can inflate the level of genetic structure observed between populations.

Indigenous, high canopy, evergreen forests are South Africa’s most restricted and naturally fragmented biome (Eeley et al. 1999; Mucina & Geldenhuys 2006) covering only 0.4% of the country’s land surface area (Berliner 2005). Given that forests have the highest biodiversity per unit area of any biome in South Africa (Berliner 2005) the extent to which habitat fragmentation impacts on forest animal species at the population genetic level (on a landscape scale) has received comparatively little attention (Eggert et al. 2008; Madisha et al 2017). Our study focuses on a population of South Africa’s only diurnal, forest dwelling primate species, the samango monkey (*Cercopithecus albogularis schwarzi*), whose distribution pattern mirrors that of South African forests. The species is nationally listed as Vulnerable and the subspecies *C. a. schwarzi* found in the study area as Endangered (Linden et al. 2016). In this study, we use microsatellite and mitochondrial DNA (mtDNA) data to investigate how historical and recent forest habitat fragmentation impacts on the genetic diversity and structure of a samango monkey population in a longitudinal (running lengthwise from east to west) mountain range, the Soutpansberg, in far northern South Africa. Forest fragmentation in the Soutpansberg is a result of both natural processes as well as anthropogenic activities (Scott 1987; Munyati & Kabanda 2008) and the matrix surrounding forest fragments is very diverse. This makes it an ideal landscape to investigate how samango monkeys are genetically impacted under various fragmentation and surrounding matrix scenarios. Being a mountain range, the study area can further be considered a biogeographic island characterised by a certain degree of isolation from its surroundings. Given the landscape characteristics, we expect that the samango monkey population is genetically subdivided within the mountain range and that gene flow between the mountain and the closest samango monkey populations further south has become very restricted. We expect that population subdivision is driven by three main processes: 1) natural habitat fragmentation driven by paleoclimatic changes 2) anthropogenic habitat fragmentation caused by land transformation and 3) geographic barriers such as distance and topographic features. With our study we aim to identify main natural and anthropogenic barriers causing population subdivision and use this to inform conservation planning for the Soutpansberg samango monkey population.

2. Materials & Methods

2.1. Study species

Samango monkeys live in multifemale groups led by a single adult male, with females being philopatric as is common in forest guenons (Cords 2001). Samango males emigrate from their natal group roughly a year before reaching sexual maturity at about 6-7 years of age (Henzi & Lawes 1987; Ekernas & Cords 2007). Extra-group males are described to range widely and interact with more than one group of females (Swart & Lawes 1996). Samango group sizes vary between 16 to 60 individuals across study sites in South Africa (Lawes 1990; Lawes 1992; Coleman & Hill 2014; Novak et al. 2014; Linden et al. 2015; Wimberger et al. 2017) as do home range sizes with 15 to 54 ha (Lawes et al. 1990; Coleman & Hill 2014; Wimberger et al. 2017). Neighbouring groups home ranges often overlap (Lawes 1992; Lawes & Henzi 1995; Novak et al. 2014) and extra-group male ranges may overlap considerably with group home ranges (Swart et al. 1993).

Using the average age of first breeding for females (Oklander et al. 2017) the generation time of samango monkeys is ~ 7 years (Cords 2012).

2.2. Study area and study population

The Soutpansberg is a ~ 210 km long and ~ 60 km wide (widest point), east-west orientated mountain range with an altitudinal range between 200 m near Pafuri in the far east and 1748 m (Lejuma) near the far west (Hahn 2017a) (Figure 1). High canopy, evergreen forests suitable for samango monkeys are only found on the southern ridges of the mountain with forest patches in the more arid western Soutpansberg being smaller and naturally more confined compared to forests in the east.

This study focussed on five samango monkey populations of which four were from the Soutpansberg *sensu stricto* (Lajuma (LA), Schoemansdal/Buzzard Mountain (SB), Levuvhu/Luonde (LL), Entabeni/Thathe Vondo (ET)) and one from the escarpment (Magoebaskloof (MK) south of the Soutpansberg (Figure 1). We chose populations across the Soutpansberg according to geographic distance from each other, matrix surrounding forests and position in relation to main barriers. The LA population is located in the far west of the mountain range, west of a main topographic feature, the north-south running Sand River gorge (Figure 1). The SB population is located east of the Sand River gorge, also in the western part of the Soutpansberg. The surrounding matrix of both western populations is composed of natural vegetation (Figure 2). The ET population is the furthest eastern population sampled and the LL population is closest to the escarpment south of the mountain. The surrounding matrix of both the LL and ET populations is characterised largely by silvicultural and agricultural areas (Figure 2). The eastern and western populations are further separated by a 30 km wide distribution gap found in the middle Soutpansberg (Linden et al. in prep.) (Figure 1). It is important to mention that the SB study area received about six individuals, including at least one male, from the ET study area as part of a Department of Water Affairs and Forestry (DWAF) translocation programme in the 1980’s (John Greaves pers. comm.). Samango monkeys were found to cause damage to pine trees in timber plantations in the ET area and the problem was managed by trapping and translocating individuals (Droomer 1985; von dem Bussche & van der Zee 1985). We included this population as the survival and successful integration of translocated individuals into existing groups was not monitored and thus their fate unknown. Samples from the MK population were included to determine the degree of differentiation and gene flow between the Soutpansberg and escarpment populations as we consider this the most likely historical migration route into the Soutpansberg. Here, a population is not considered equal to a group of samango monkeys. A certain geographic area may contain several groups of samango monkeys making up a local population in a particular forest patch and a study by Madisha et al. (2017) showed that gene flow between neighbouring samango groups was high. An attempt was made to sample different groups in study areas to avoid re-sampling of the same individuals of a local population.

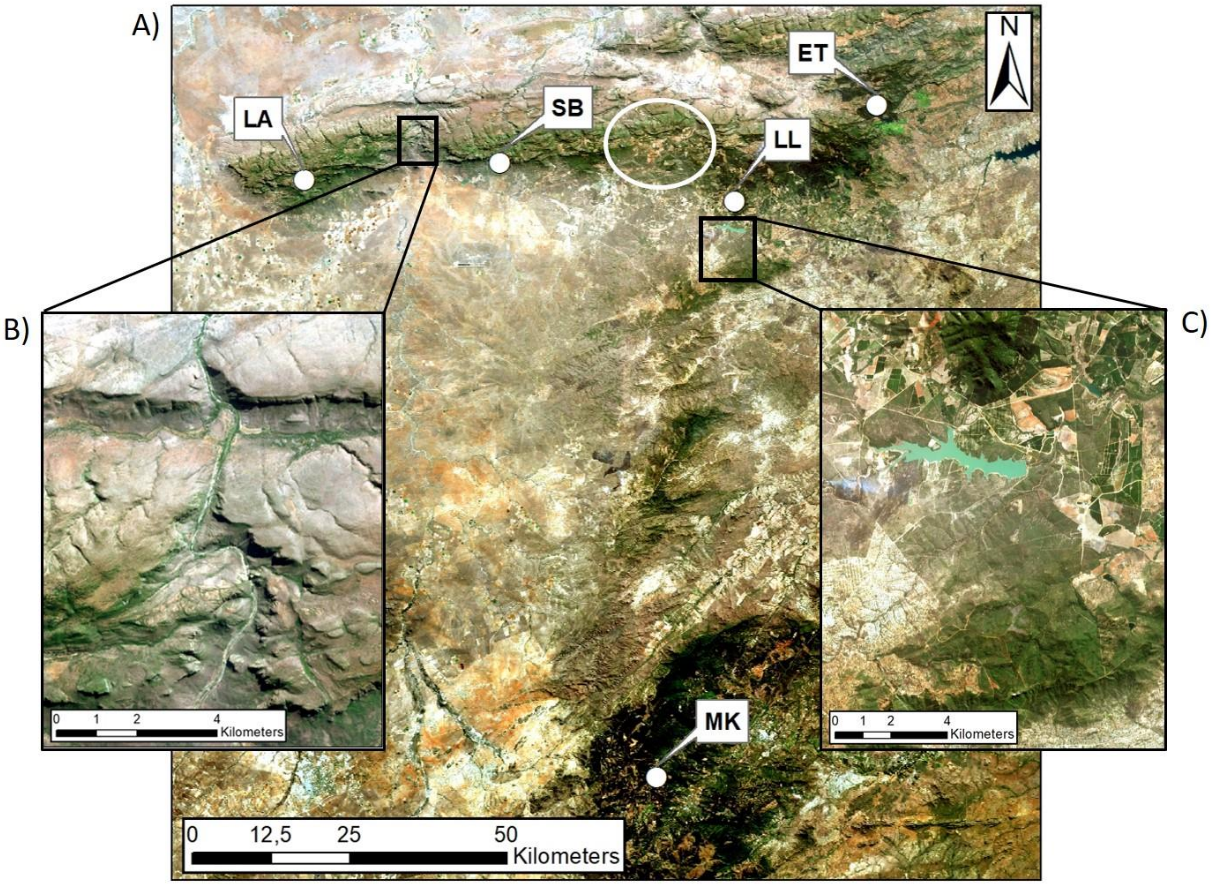

Figure 1. Geographic setting. A. Satellite image showing the five study sites across the Soutpansberg (LA, SB, LL, ET) and northern escarpment (MK). Geographic barriers are detailed in the two enlargements; the Sand River gorge (inset B) and the gap between the Soutpansberg and escarpment (inset C). The area circled in white shows the ~ 30km samango monkey distribution gap in the middle Soutpansberg. Satellite image: European Space Agency (ESA), Sentinel 2 (2019).

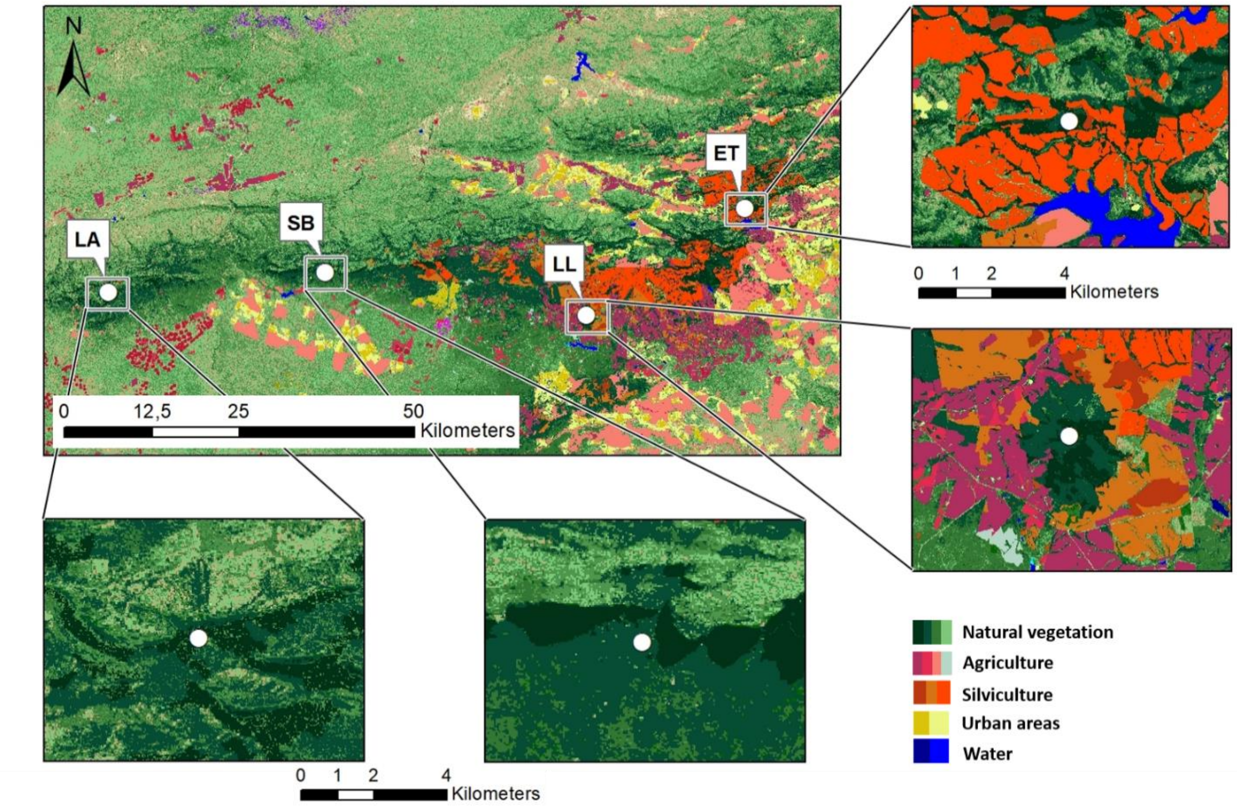

Figure 2. Land cover map showing the four sampling areas in the Soutpansberg in the context of land use/transformation. Enlarged maps for each sampling area show the matrix surrounding indigenous forests. Map data: South African national land-cover (NLC) (2014).

2.3. Sample collection and DNA extraction

2.3.1. Ethical Note

Relevant permission for collecting and storing both tissue and faecal samples was obtained (Limpopo Provincial Government (Republic of South Africa), Department of Economic Development, Environment & Tourism, Permit Code 0089-MKT001-00002; Department of Environmental Affairs (Republic of South Africa) Standing Permit No. S07507). For samples collected on private land, landowner permission was acquired. For samples collected in national forests relevant permits were sourced (Komatiland Forests, License Number: 19017 and License Number: 19023).

2.3.2. Faecal samples

We collected 29 faecal samples between 2012 - 2015 from the four geographic areas across the Soutpansberg: LA, SB, LL and ET (Table 1). We sampled two samango monkey groups per area (Table 1). When collecting the samples in the field, strict precautions were used to avoid contamination (Goossens et al. 2003). When possible, faecal samples were collected immediately after defecation to obtain high quantity and quality DNA and avoid degradation (Wasser et al. 1997). However, this was not always possible in the field as all but one population (LA) sampled were completely unhabituated. Sample collection on the habituated groups in LA was achieved by following the samango monkeys (following distance could be < 5 m), observing for any individual defecating and collection of samples within 5 min. For all other groups sampled, following distances were greater, making observations of individuals defecating and detecting faeces on the forest floor more difficult. Thus, this resulted in samples being collected between 5 to 60 min after defecation. As most shed cells are found at the 'front end' and the outside of faeces as much from the outside of the material as possible was collected to maximize DNA yield (Goossens et al. 2003). Faecal samples were directly transferred into absolute ethanol (sample:ethanol 1:3) (Gerloff et al. 1999) and then stored in a refrigerator no warmer than 4 °C (Goossens et al. 2003). Samples were processed in the lab within four weeks of collection. DNA from faecal samples was extracted using ZR Faecal DNA MiniPrep^{TM} (Zymo Research) extraction kit using the manufacturer's recommendations.

2.3.3. Tissue samples

Tissue samples were collected opportunistically from 2012 - 2015 in one locality (LL population) where samango monkeys regularly get killed through car collisions (Linden et al. in press) (Table 1). The total number of groups sampled through roadkills could not be established from the individuals found. As the particular stretch of road was driven several times a day, roadkills were detected relatively quickly after they occurred. Additionally, one samango carcass was sampled from an individual in Magoebaskloof (MK) which was reportedly found dead (possibly killed by an eagle) on a private property. Tissue samples from all 12 individuals were taken from the muscle and ear (skin and cartilage) and immediately stored in absolute ethanol in a refrigerator (4 °C). Samples were processed in the lab within four weeks of collection. Extraction of DNA from tissue samples was conducted using the ZR Genomic DNA^{TM} Tissue MiniPrep (Zymo Research) following the extraction protocol as outlined by the manufacturer. Another 20 tissue samples from Dalton et al. (2015) were included in the analysis of this study: 15 for LA and five for MK (Table 1).

2.4. Microsatellite genotyping

All samples were initially genotyped for polymorphism at the 21 microsatellite loci used for samango monkey tissue samples in Dalton et al (2015) following methods described therein. Faecal DNA amplification was carried out using a 15 micro litre (µl) reaction volume and polymerase chain reaction (PCR) was conducted with 2 X Platinum Multiplex PCR Master Mix (Life Technologies^{TM}). The final reaction conditions were as follows: 1 X Master Mix, 10 pico-mol (pmol) of each of the forward and reverse primer, 10–20 nano gram (ng) genomic DNA template. The conditions for PCR amplification were as follows: 5 min at 95 °C initial denaturation, 30 cycles for 30 seconds (sec) at 95 °C, 30 sec at 50 °C and 1 min at 72 °C, followed by extension at 72 °C for 20 min. The PCR reaction was carried out in a T100^{TM} Thermal Cycler (Bio-Rad Laboratories, Inc.). PCR products were pooled together and run against Genescan™ 500 LIZ$^{®}$ internal size standard on an ABI 3500 Genetic Analyzer (Applied Biosystems, Inc.). Samples were genotyped using GeneMapper software version 4.0 (Applied Biosystems, Inc.). Due to lower DNA quantity and quality obtained from faecal samples, amplification was repeated three times (Goossens et al. 2003) and every faecal sample missing data for 50% or more loci was excluded from further analysis. Thus, the final microsatellite dataset included 61 tissue and faecal samples from five different samango populations (Table 1). As we used both tissue and faecal samples in this study, the quality of DNA extraction and amplification from faecal samples could be directly assessed through comparison to tissue sample DNA products. Of the 21 markers initially genotyped for polymorphism, 13 (D4S243, D12S67, D9S922, D3S1768, D8S1106, D15S108, D1S518, D18S536, D10S1432, D11S925, D13S765, D5S1475, D1S207) were polymorphic and gave consistent results for both tissue and faecal samples.

Table 1. Number of samango groups sampled, total number of samples and sample types from all five study areas, listed from west (top) to east (bottom) within the Soutpansberg and the outgroup from the escarpment (Magoebaskloof).

study areas	groups sampled	sample number	sample type
Lajuma (LA)	2	22	7 faecal^1, 15 tissue^2
Schoemansdal/Buzzard Mountain (SB)	2	13	faecal^1
Levuvhu/Luonde (LL)	N/A	13	11 tissue^1, 2 faecal^1
Entabeni/Thathe Vondo (ET)	2	7	faecal^1
Magoebaskloof (MK)	2	6	tissue^{1,2}
TOTAL		**61**	**32 tissue, 29 faecal**

^{1} = Samples from this study
^{2} = Samples included from Dalton et al. (2015)

2.5. Microsatellite analysis

2.5.1. Genetic diversity

MICRO-CHECKER was used (Van Oosterhout et al. 2004) to detect potential genotyping errors, allelic dropout and non-amplified alleles (null alleles) for each microsatellite locus within our dataset. Linkage disequilibrium (LD) between pairs of microsatellite loci within each population, deviations from Hardy-Weinberg equilibrium (HWE) and the fixation index (F_{IS}) were calculated using ARLEQUIN version 3.5.1.2 (Excoffier and Lischer, 2010). To estimate genetic diversity within populations, the mean number of alleles per locus (N_A), observed heterozygosities (H_O), expected heterozygosities (H_E) and unbiased expected heterozygosity (uH_E) was determined using GenAlEx version 6.5 (Peakall and Smouse, 2012). Allelic richness (Ar) was estimated correcting for sample size through rarefaction using HP-RARE v. June-6-2006 (Kalinowski 2005). Pairwise relatedness analysis was conducted in GenAlEx 6.5.

2.5.2. Genetic structure

To assess overall population structure two approaches were used, namely: a model-based Bayesian clustering algorithm implemented in STRUCTURE version 2.3.4 (Pritchard et al. 2000) and a non-model-based discriminate analysis of principal components (DAPC) to analyse cryptic structure (Jombart 2010). STRUCTURE was used to determine the most probable number of populations and to assign individuals to their most likely population of origin. STRUCTURE was run with a model assuming admixture, without any prior population information and with correlated allele frequencies. We used a burn-in period of 100 000 followed by 700 000 repetitions of MCMC. All the runs were replicated ten times for K = 1 -

10. The K with the greatest increase in posterior probability (ΔK) (Evanno et al. 2005) was identified as the optimum number of sub-populations using STRUCTURE HARVESTER (Earl 2012). The DAPC was performed using the R package adegenet version 3.1.9 (Jombart 2008). Here, the most likely number of clusters (between 1 and 30) associated with the lowest Bayesian Information Criterion (BIC) was determined using the find.cluster function in adegenet 3.19. Optimization α-score analysis determined that seven principal components should be retained for assignment (Figure S.1), thus the DAPC was performed using the dapc function in adegenet retaining seven principal components. We determined population relatedness by calculating hierarchical *F*-statistics across a range of population grouping scenarios in an AMOVA framework (Excoffier at al. 1992). The scenario with the highest among-population variation (F_{ST}) thus describes the most likely pattern of population relatedness. We also explicitly tested Wright's (1943) model of isolation by distance, to determine the role played by geographic distance in shaping the observed genetic structure. This was achieved by a Mantel test (Mantel 1967) between matrices of pairwise F_{ST} and geographic distance in ARLEQUIN version 3.5.1.2 (Excoffier & Lischer 2010). Euclidean (straight-line) distances between populations were determined in ArcGIS version 10.5 (Esri®), taking the centre of each sampling site. Analyses of Molecular Variance (AMOVA, Excoffier et al. 1992) was conducted in ARLEQUIN version 3.5.1.2 (Excoffier & Lischer 2010) to determine the distribution of genetic variation in the data set.

2.5.3. Inference of recent migration rates

To estimate the impact on samango monkey dispersal of recent anthropogenic activity (land transformation), contemporary levels of gene flow between each population were inferred using BIMr (Bayesian inference of migration rates, Faubet & Gaggiotti 2008). For this analysis Markov Chain Monte Carlo (MCMC) sampling was implemented to determine a posterior estimate of the pair-wise migration matrix. BIMr was run with a MCMC of 10 million, a burn-in of 2 million, thinning interval of 10 000 and a sample size of 1 000. We performed the analysis using the F-model as we assume population admixture before the last generation of migration and as it has shown to improve MCMC conversion when population differentiation is weak. We performed five independent runs with eight repeats each, resulting in a total of 40 migration rate estimates for each pair.

3. Results

3.1. Genetic diversity

MICRO-CHECKER detected null alleles for one locus (D4S243) in the LA population, one locus (D13S765) in the SB population, two loci (D12S67, D10S1432) in the LL population and one locus (D1S207) in the MK population. These markers additionally deviated from HWE in the respective populations. Evidence of linkage disequilibrium following Bonferroni correction was detected between 12 marker pairs in the LA population, between one marker pair in the SB group, between two marker pairs in the LL group and between three markers pairs in the ET group. Null alleles, deviations from HWE and linkage disequilibrium were observed to be randomly distributed across locus pairs in the various populations tested. Thus, the data generated using these markers were considered valid to assess genetic structure between samango monkey populations. For the five populations reflecting sampling areas, genetic diversity estimates were similar with the mean number of alleles (N_A) and the expected heterozygosity (H_E) across loci and populations being 3.37 and 0.53 respectively (Table 2). N_A was highest in the LL population (3.54) and lowest in both the SB and ET populations (3.15). Expected heterozygosity was highest in the MK (0.56) population and lowest in the LA (0.48) population. Variability of SB ($H_E = 0.53$) and LL ($H_E = 0.54$) was similar and second lowest in ET ($H_E = 0.51$). Unbiased expected heterozygosity (uH_E) ranged between 0.49 and 0.62. Results show that observed heterozygosity (H_O) was lower than H_E in all populations sampled (Table 2). Further analysis using a two tailed pairwise t-test ($\alpha = 0.025$) showed this difference to be significant in the ET population but not in LA, SD, LL and MK. Private alleles were observed in all five populations: eight for ET, six for LL, five for MK, four for LA and one for SB. F_{IS} was highest in the ET population (0.35, $P = 0.007$) followed by LL (0.16, $P = 0.054$) and LA (0.13, $P = 0.057$). The other two populations (SB, MK) showed slightly negative F_{IS} values (Table 2, $P = 0.699$ and 0.815 respectively). Pairwise relatedness was higher between individuals within local populations (average relatedness varied from 0.097 to 0.4359) than between individuals among different local populations (average relatedness varied from -0.198 to 0.0621) (Table S.1). The LA population had the highest average within population relatedness (0.4359), followed by SB (0.1669), LL (0.1693) and ET (0.1313). The MK population displayed the lowest average within population relatedness (0.097). Between populations, the overall highest relatedness was observed between ET and SB (0.0621) with all other relationships showing negative values.

Table 2. Genetic variation estimates across all populations (LA = Lajuma, SB = Schoemansdal/Buzzard Mountain, LL = Levuvhu/Luonde, ET = Entabeni/Thathe Vondo, MK = Magoebaskloof) with the number of alleles (N_A±SE), allelic richness (Ar), observed (H_O±SE) and expected (H_E±SE) heterozygosities, unbiased expected heterozygosity (uH_E±SE) and inbreeding coefficient (F_{IS}) over all loci.

Population	**N_A**	**Ar**	**H_O**	**H_E**	**uH_E**	**F_{IS}**
LA	3.54±0.46	2.02	0.46±0.06	0.48±0.06	0.49±0.06	0.13
SB	3.15±0.32	2.16	0.52±0.07	0.53±0.05	0.56±0.05	-0.04
LL	3.77±0.3	2.21	0.46±0.07	0.54±0.05	0.56±0.05	0.16
ET	3.15±0.32	2.23	0.27±0.07	0.51±0.05	0.58±0.06	0.35*
MK	3.23±0.28	2.32	0.54±0.09	0.56±0.04	0.62±0.04	-0.12
Total (mean±SE)	**3.37±0.15**	**2.19**	**0.45±0.03**	**0.53±0.02**	**0.56±0.02**	**-**

*** = P < 0.05**

3.2. Genetic structure

Our preliminary analyses of population structure using model-based (STRUCTURE) and non-model-based (DAPC) algorithms indicated the existence of genetically distinct units of samango monkeys. STRUCTURE HARVESTER identified the most likely number of populations as K = 3 (Figure 4a and b) with the following grouping of individuals: (1) LA, (2) SB and ET, (3) LL and MK. However, as the K reported by STRUCTURE represents the uppermost genetic hierarchical level, not necessarily the best K, we compared STRUCTURE results for K = 2 to K = 5 to the DAPC and AMOVA results.

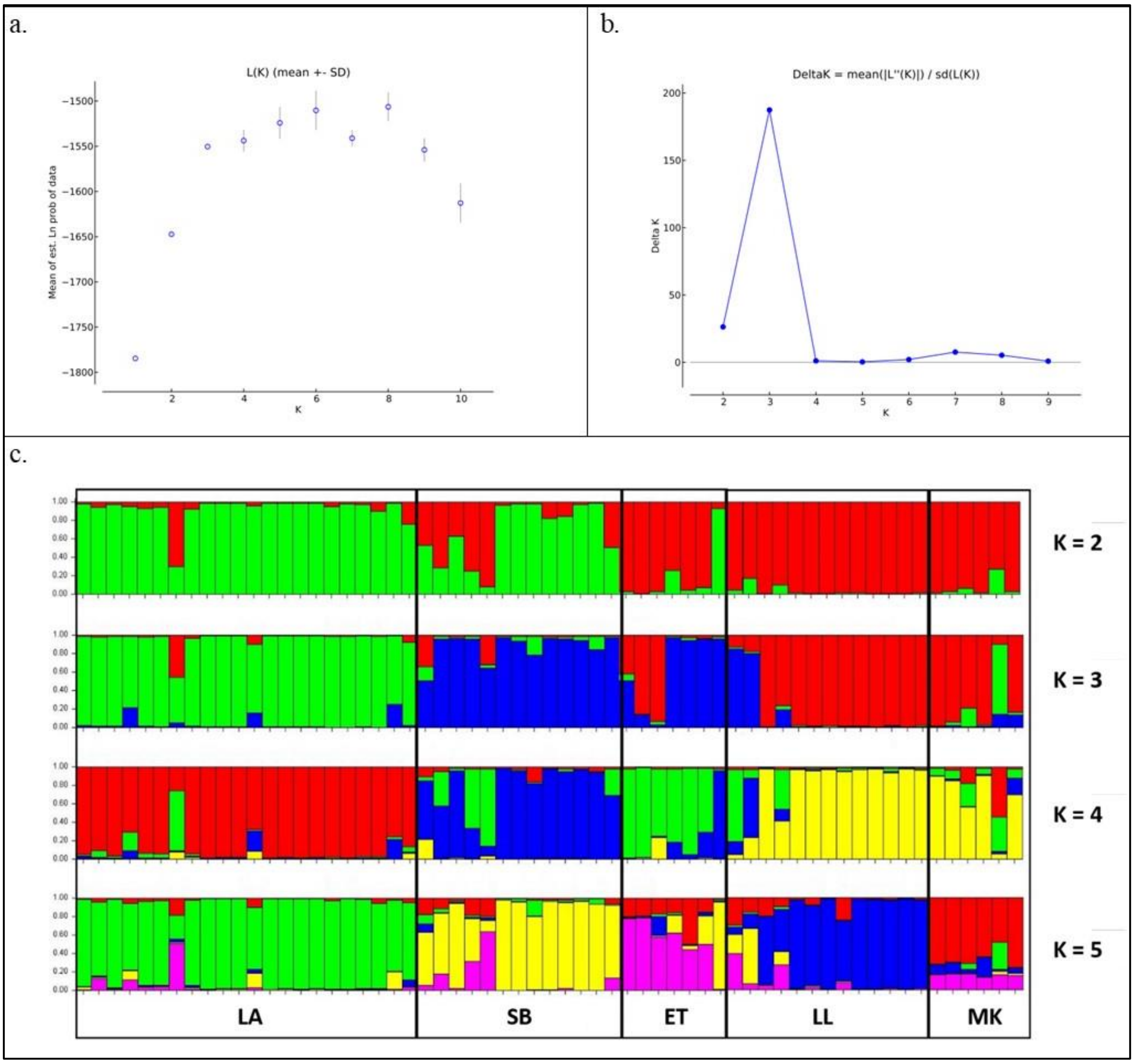

Figure 3. Top panels (a and b) show the mean log likelihood Ln P(X|K) and DeltaK as a function of the number of genetic clusters (K) averaged over 10 consecutive STRUCTURE runs for each K (error bars indicate one standard deviation) with (a) Probability (-LnPr) of K = 1-10, (b) Delta K values for real population structures of K = 1-10. Bottom panel (c) shows Bayesian assignment probabilities for K = 2 to K = 5 of microsatellite genotypes. Each individual is represented by a single vertical bar, with lengths proportional to the estimated membership in each cluster. LA = Lajuma, SB = Schoemansdal/Buzzard Mountain, ET = Entabeni/Thathe Vondo, LL = Luonde/Levuvhu and MK = Magoebaskloof.

Although STRUCTURE provided support for the genetic differentiation of three populations, the hierarchical population structure inferred using DAPC supports five genetic clusters (Figure 5 a and b; Figures S.1 and S.2). The two main axes of the DAPC analysis explained 99.29% of the total variability among clusters. The presence of these five genetic populations in the dataset through the DAPC analysis was further confirmed based on AMOVA (Table 3). In both cluster scenarios (STRUCTURE and DAPC), there were a few outlier individuals that were collected in one locality but were placed in different genetic cluster based on genetic data. Results from the AMOVA analysis across the samango monkey populations demonstrated the highest proportion of variation being observed when populations are separated into five groups namely LA, SB, LL, ET and MK (Table 3).

Table 3. Analyses of Molecular Variance (AMOVA) when considering various population grouping scenarios. Values indicated in bold provide support for the grouping that displays the highest among group variation and the lowest among population variation.

Scenario	**Percentage variation within individual (P-value)**	**Percentage variation among individual within population (P-value)**	**Percentage variation Among population (P-value)**
1 group, 2 populations (LA, SB-ET-LL-MK)	67.77 (<0.001)	19.81 (<0.001)	12.42 (<0.001)
1 group, 2 populations (LA-SB-ET-LL, MK)	67.98 (<0.001)	23.68 (<0.001)	9.33 (<0.001)
1 group, 3 populations (LA, SB-ET, LL-MK)	68.68 (<0.001)	15.57 (<0.001)	15.76 (<0.001)
1 group, 3 populations (LA-SB, ET, LL-MK)	67.75 (<0.001)	17.47 (<0.001)	14.78 (<0.001)
1 group, 3 populations (LA-SB, ET-LL, MK)	68.20 (<0.001)	18.38 (<0.001)	13.42 (<0.001)
1 group, 4 populations (LA, SB, ET, LL-MK)	68.77 (<0.001)	14.07 (<0.001)	17.16 (<0.001)
1 group, 5 populations (LA, SB, ET, LL, MK)	**69.14 (<0.001)**	**12.22 (<0.001)**	**18.64 (<0.001)**

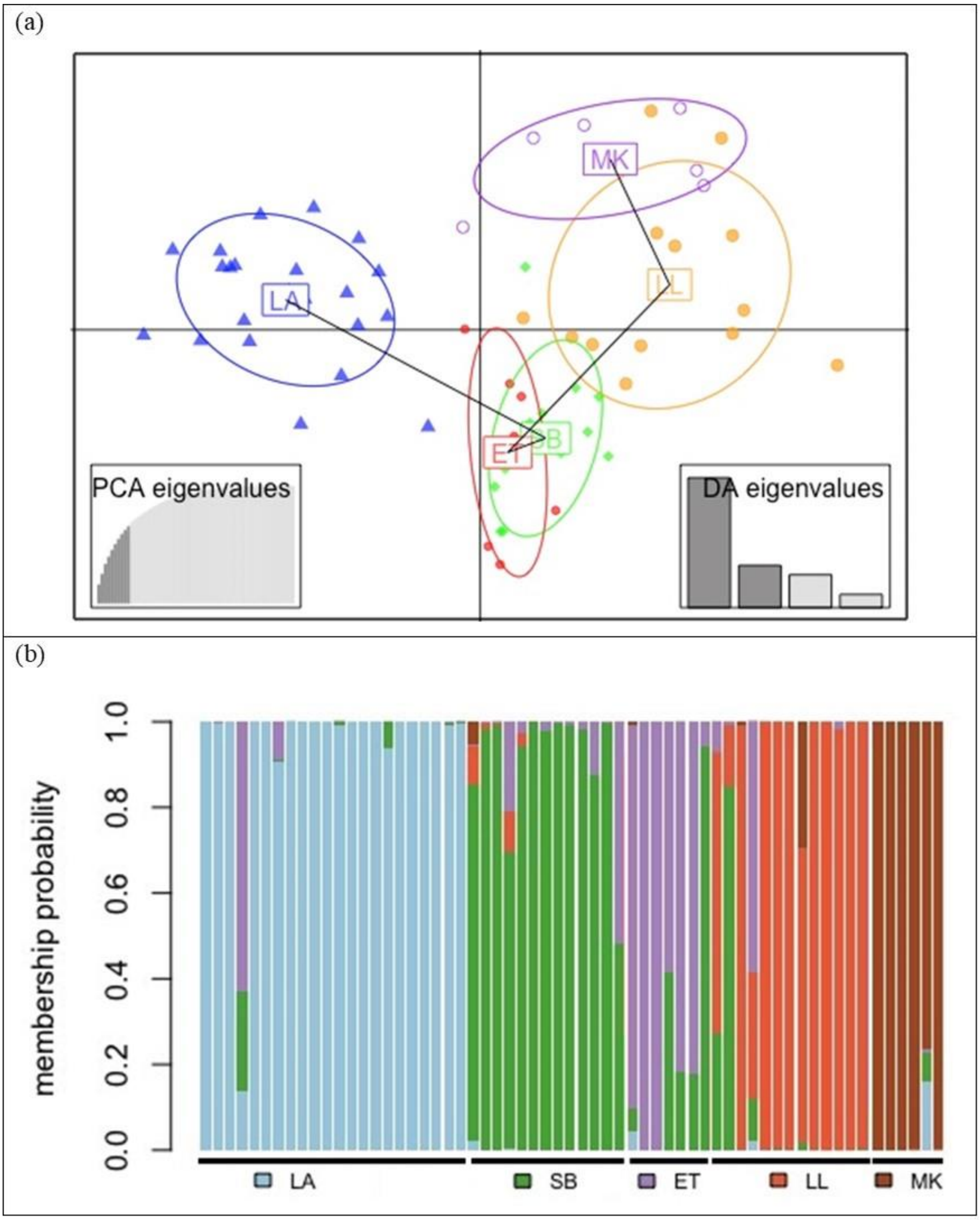

Figure 4. (a) Discriminant analysis of principal components (DAPC) of samango monkeys grouped into five clusters. Each point represents an individual, shapes show site of origin of an individual and colours indicate the assignment of individuals to clusters. (b) Population structure of samango monkeys provided by DAPC. Colours represent different assigned clusters and each individual is represented by a single vertical bar. LA = Lajuma, SB = Schoemansdal/Buzzard Mountain, ET = Entabeni/Thathe Vondo, LL = Luonde/Levuvhu and MK = Magoebaskloof.

Microsatellite-based pairwise F_{ST} values and associated P-values are indicated in Table 4. Moderate to high genetic differentiation was observed between all populations ($F_{ST} = 0.048$ – 0.252), with an average of 0.18642 ($P < 0.001$). In general, populations that were geographically closer displayed lower pairwise F_{ST} values. Exceptions here are the ET and LL groups that are geographically close (27.35 km) with a higher F_{ST} value (0.134) and the SB and ET groups that are moderately geographically distant (59.36 km) but show a very low F_{ST} value (0.074). The straight-line distance from the furthest east to the furthest west population sampled was 90.45 km (LL to ET) and the shortest distance between Soutpansberg and escarpment populations was 91.83 km (LL to MK) (Table 4). The Mantel test showed a significant correlation between genetic distance and geographic distance ($r = 0.6744$, $Z = 106.84$, $P = 0.002$) with 45% ($r^2 = 0.4548$) of genetic difference being explained by distance (Figure S.3).

Table 4. Comparison of geographic distance (in km, below diagonal) and population differentiation (microsatellite-based pairwise F_{ST} values and associated P-values estimated during AMOVA, above diagonal) between all five sampling areas: Lajuma = LA, Schoemansdal/Buzzard Mountain = SB, Levuvhu/Luonde = LL, Entabeni/Thathe Vondo = ET, Magoebaskloof = MK.

Population	**LA**	**SB**	**LL**	**ET**	**MK**
LA	/	0.048 **(<0.001)**	0.118 **(<0.001)**	0.117 **(<0.001)**	0.189 **(<0.001)**
SB	30.44	/	0.114 **(<0.001)**	0.074 **(<0.001)**	0.171 **(<0.001)**
LL	67.66	37.13	/	0.134 **(0.009)**	0.101 **(<0.001)**
ET	90.45	59.36	27.35	/	0.252 **(<0.001)**
MK	109.44	100.3	91.83	108.52	/

3.3. Inference of recent migration rates

MCMC trace plots of each pair-wise estimate were checked and runs with poor MCMC convergence were excluded (Faubet & Gaggiotti 2008). We used pair-wise migration estimates of the five best runs to calculate the average migration rate (Robin et al. 2015). Our results show that overall recent migration rates between populations were so low (2.35E-11 – 5.83E-11) that they can be considered 0 (Table S.2).

4. Discussion

4.1. Genetic diversity

Genetic diversity across all five sampled populations was similar regarding allelic richness (Ar between 2.32 and 2.02) and heterozygosity (H_E between 0.48 and 0.56). For the ET population we found that the H_O was significantly lower than the H_E, indicating inbreeding. In the wild, a deficiency of heterozygotes can be caused by the Wahlund effect (Wahlund 1928) likely due to a mix of two sub-populations that mate mostly among themselves but overlap spatially. The lowest heterozygosity and Ar were observed in the LA population (H_E 0.48) in the far western part of mountain range. On the other hand, the highest genetic diversity values were observed in the MK population (H_E 0.56) from the escarpment south of the Soutpansberg. Our results show that heterozygosity of populations within the Soutpansberg decreases with increasing distance to the escarpment population and from east to west. When accounting for variation in sample size across populations (uH_E and Ar) comparable results were obtained.

When investigating the loss of heterozygosity across populations due to possible inbreeding, LL and LA populations showed similar, non-significant inbreeding coefficients ($F_{IS} = 0.16$, $P = 0.054$ and $F_{IS} = 0.13$, $P = > 0.057$), while the inbreeding coefficient result for the ET population was statistically significant ($F_{IS} = 0.35$, $P = 0.007$). Although positive F_{IS} values may indicate inbreeding, they can also be caused by null alleles (Brookfield 1996), unrecognized fine scale structure (Hartl & Clark 1987) or small sample size. Null alleles were absent in the ET population. The MK and SB populations showed an absence of inbreeding, with slightly negative inbreeding coefficients ($F_{IS} = -0.12$ and -0.04). A negative F_{IS} value indicates that there is more heterozygosity than would be expected under Hardy Weinberg equilibrium. This may occur due to recent outbreeding events but can also be the result of random sampling errors or small sample size. As would be expected from a female phylopatric, group living species, our results show that average relatedness within local populations was higher than between populations particularly within the LA population. Although we sampled two groups per geographic location (forest patch), neighbouring groups may be “sister-groups” due to group fission (e.g. when initial group grew too large) resulting in related individuals across groups (Swart & Lawes 1996; Linden pers. obs.).

4.2. Historic connectivity

The Last Glacial Maximum (LGM) at 21 000 BP resulted in a substantial contraction of forest cover in southern Africa due to much drier climatic conditions (Deacon 1983). Pollen records and radiocarbon dating of peat deposits in the Thathe Vondo area in the Soutpansberg suggest that at around 12 000-10 000 years BP forests were well developed in mountain ravines and under south-facing cliffs and that slopes were largely covered by open vegetation (grassland and fynbos elements) (Scott 1987). From between 10 000-6 500 years BP this same study found a reduction in forest elements and increase in savanna elements and from 6 500 years onwards, more swamp and mesic woodland elements suggest relatively moist conditions. These paleohistoric changes in forest extent and connectivity will have shaped the distribution and connectivity of samango monkey populations in the Soutpansberg.

Overall, pairwise fixation indices and isolation by distance results followed a similar pattern as observed for the genetic diversity of populations, with the highest differentiation observed between populations geographically furthest apart (> 100 km) namely between LA and MK ($F_{ST} = 0.189$) and ET and MK ($F_{ST} = 0.252$). Results from the isolation by distance analysis showed a significant correlation between genetic differentiation and geographic distance. The SB population showed results contrary to expectations with the weakest differentiation observed between it and the ET population ($F_{ST} = 0.074$) and the lowest average within population relatedness despite geographic distance (59 km). We suggest that this result could be explained by the recent translocations from ET individuals to the SB population (John Greaves pers. comm.). The LA population is the only population sampled occurring west of a major topographic feature, the Sand River gorge (Figure 1). We could however not find any evidence that this gorge in particular poses a barrier to samango monkeys as the pairwise F_{ST} value between LA and its closest neighbour SB is low compared to other F_{ST} values and, rather, in line with what is expected from isolation by distance (Table 4). An interesting result is of that between LL and ET as the differentiation between them is higher than expected ($F_{ST} = 0.134$) by geographic distance (27 km) suggesting that other landscape related processes such as anthropogenic practices in the matrix surrounding forest fragments are involved in the genetic structuring of populations.

4.3. Contemporary connectivity

Humans first settled in the mountain range from around AD 300 onwards (Eastwood & Eastwood 2006) and Scott (1987) showed a sharp decline in tree pollen at around 1 500 BP and suggested that this was linked to the burning and clearing of woody vegetation by the first Iron Age people in the area. The arrival of people of European descent saw the beginnings of commercial silviculture in the late 1800's and the expansion and commercialisation of agriculture in the early 1900's (Scholes et al. 1995; Tempelhoff 1999). As indicated, anthropogenic land use and land transformation vary markedly between the eastern and western Soutpansberg.

Both the LA and SB populations from the west occur in areas where much of the matrix surrounding forests is composed of indigenous and supposedly natural vegetation (Figure 2), namely Soutpansberg Mountain Bushveld (Mucina & Rutherford 2006). However, much of this woodland/thicket vegetation type is considered novel having established (due to cumulative effects of anthropogenic activities) from the 1920's onwards in grassland areas not utilised for silviculture and agriculture (Hahn 2017b). As samango monkeys have been recorded to utilise elements of this vegetation type in daily foraging sorties (Linden et al. 2015), we do not consider it to be a distribution barrier. Here we suggest that geographic distance between suitable forest patches continues to play a main role in the isolation of populations in the western Soutpansberg.

Contrary to this, the LL and ET populations in the eastern part of the Soutpansberg are surrounded by extensive commercial silvicultural and agricultural areas (Figure 2). These two populations showed a higher genetic differentiation than would be expected by geographic distance and the ET population showed the highest inbreeding index of all studied populations. Although it has been shown that samango monkeys utilise timber plantations to some degree (Droomer 1985; von dem Bussche & van der Zee 1985), the distance of 27 km between them can obviously not be overcome. Further, when samangos enter the surrounding matrix their exposure to threats linked to human proximity (e.g. roads, domestic dogs, power lines) rapidly increases, potentially hampering successful dispersal (Linden at al 2016; Linden et al. in press). Regarding the connectivity between the escarpment and the Soutpansberg, old missionary records from the Levuvhu area at the foothills of the Soutpansberg show evidence of a once far more extensive lowland (riverine) forest (along the Levuvhu river and its tributaries draining out of the southern slopes) (Gründler 1897), offering a migration corridor for forest fauna. However, due to more recent anthropogenic landscape change the geographic gap between the Soutpansberg and escarpment (Figure 2a) likely poses much more of a barrier today than it did historically.

Extensive anthropogenic changes to the landscape in the eastern and distance between high canopy forest patches in the western Soutpansberg appear to have reduced the ability of samango monkeys to disperse between sampling areas. This was evident from analyses of population structure (Table 3, Figure 5) as overall the most likely scenario was five distinct populations, corresponding to each sampling area. This was further corroborated by negligibly low inferred bi-directional migration rates among all populations, suggesting little to no contemporary gene flow (Table S.2). Given an overall significant pattern of isolation by distance, it is clear that that populations along the mountain range were more connected and less distinct in the past, and that in the east a recent cessation of gene flow due to anthropogenic landscape changes, has led to greater genetic drift and in some (ET population) to potential inbreeding, which has increased population distinctiveness.

5. Conclusion

Although samples sizes of some populations were low (MK 6 and ET 7 individuals), this study provides the first analysis of genetic diversity and structure in the Soutpansberg samango monkey populations. The observed population structuring is likely a result of historical (driven by paleoclimatic change) and contemporary (driven by human landscape change) habitat fragmentation and geographic distance. Our study shows that natural and anthropogenic fragmentation and geographic distance are potential drivers for genetic differentiation observed in the Soutpansberg samango monkey population and that the matrix surrounding forests and their suitability for samango utilisation plays a role on the local scale. The degree of samango monkey population subdivision and the apparent lack of contemporary migration between populations found in our study raises concerns about the long-term viability of populations across the mountain. Those in the eastern part of the mountain being of particular concern due to intense anthropogenic pressure and those in the west likely being entirely cut off from the eastern part of the Soutpansberg and the escarpment to the south-east, leaving the western populations without any rescue effect possibilities in future. Our results further suggest that translocations of individuals from ET to SB in the 1980’s seem to have been successful as the genetic differentiation between these populations is much lower than would be expected by geographic distance. Given the longevity of samango monkeys (~ 30 years, Cords 2012), we consider it highly unlikely we randomly sampled original individuals translocated 30 years ago suggesting that individuals successfully integrated into existing groups and reproduced.

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Supplementary

Table S.1. Pairwise average relatedness coefficients within and between populations (LA, SB, LL, ET, MK) with a heat map showing highest and lowest relatedness in colour.

	LA	SB	LL	ET	MK
LA	0,4359	-0,04	-0,198	-0,192	-0,067
SB	-0,04	0,1669	-0,15	0,0621	-0,181
LL	-0,198	-0,15	0,1693	-0,049	-0,036
ET	-0,192	0,0621	-0,049	0,1313	-0,17
MK	-0,067	-0,181	-0,036	-0,17	0,097

🟥 **High relatedness**

🟩 **Low relatedness**

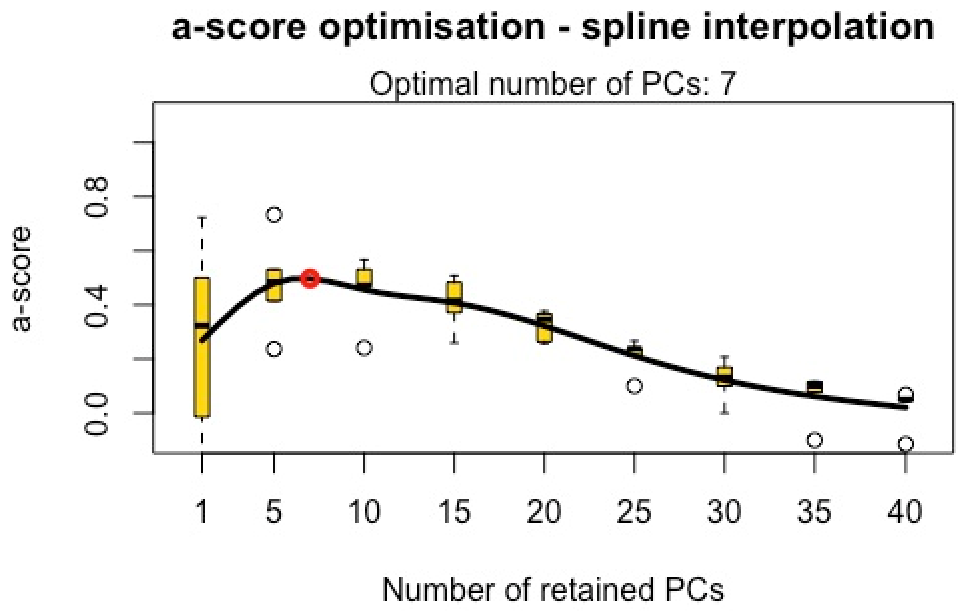

Figure S.1. The optimal number of principal components found for the analysis was 7, based on the trend in alpha scores.

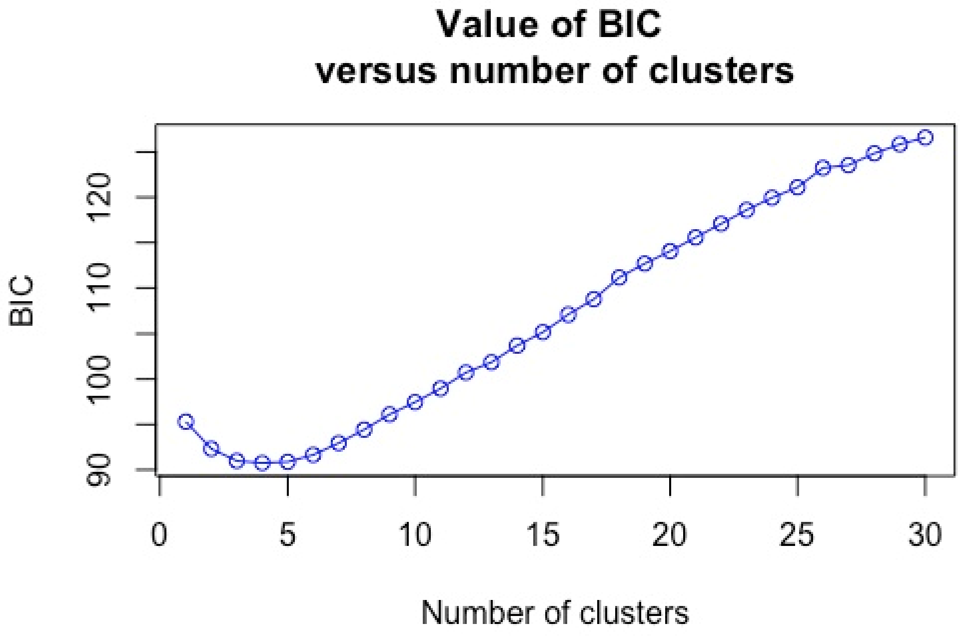

Figure S.2. Bayesian Information Criterion (BIC) values shown in relation to the number of genetic clusters.

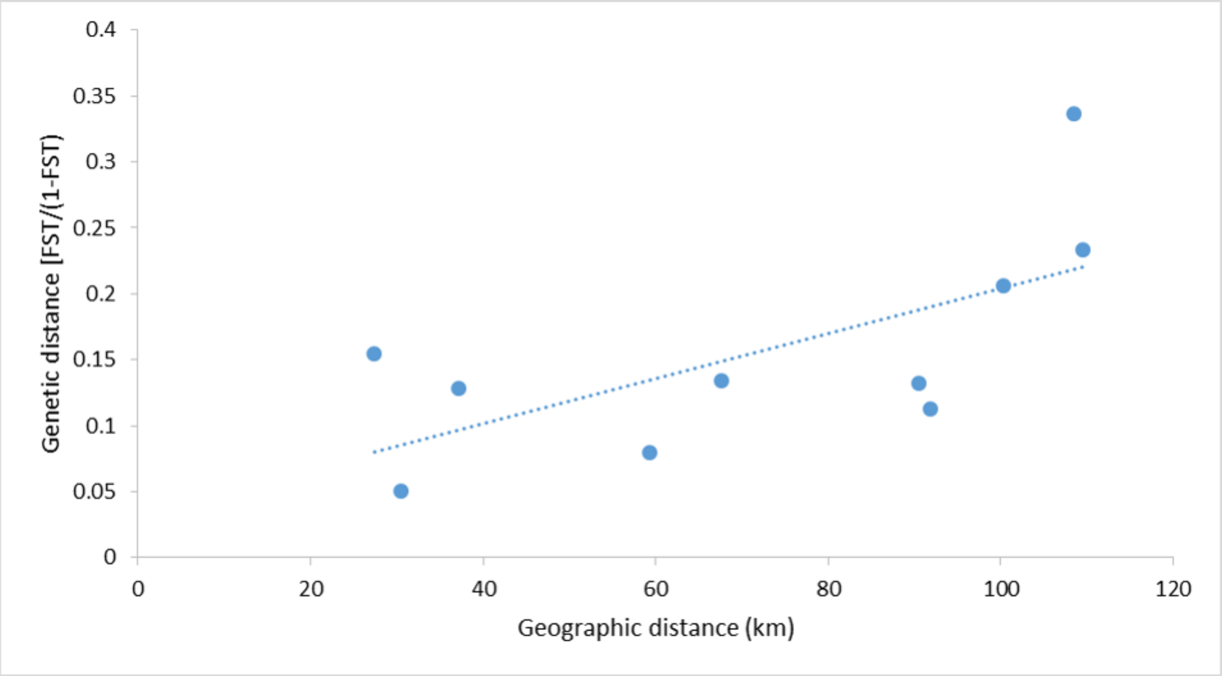

Figure S.3. Relationship between genetic distance (transformed F_{ST}) and geographic distance (km) for the five samango monkey populations.

Table S.2. Bayesian estimates of recent migration rates (BIMr) across the five samango monkey populations. Migration rates are anywhere from 0 to 1, with 0 indicating that no recent migration occurred.

Into/From	**LA**	**SB**	**LL**	**ET**	**MK**
LA	1	4.28E-11	4.29E-11	4.27E-11	4.28E-11
SB	5.78E-11	1	5.77E-11	5.79E-11	5.83E-11
LL	4.2E-11	4.16E-11	1	4.19E-11	4.19E-11
ET	2.38E-11	2.35E-11	2.38E-11	1	2.38E-11
MK	4.73E-11	4.74E-11	4.76E-11	4.73E-11	1

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Chapter 4: Bridging the gap: how to design canopy bridges for arboreal guenons to mitigate road collisions

Abstract

Roads affect the integrity of ecosystems worldwide as a cause of mortality to animals and a barrier to animal movement, decreasing gene flow and increasing local extinction probability. It is estimated that construction of linear infrastructure impacts up to 13% of primate species but research focusing on primate road fatalities and mitigation is not extensive and experimental research on canopy crossing designs for primates is lacking. We used the South African samango monkey (*Cercopithecus albogularis*) as a model species to test suitable bridge design through field experimentation and behavioural data collection for arboreal guenon roadkill mitigation. Analysis of actual roadkill data provided insights into the nature of high-risk localities. We show that canopy overpasses are a viable intervention for mitigating arboreal guenon road fatalities, reducing the probability that monkeys will cross a road on the ground. Samango monkeys clearly preferred a pole bridge over a rope ladder design and canopy bridges were preferred to trees and the ground when the tree canopy was open. Pole bridges were also used by other non-guenon and non-primate species. Although samango roadkills are not predictable in time (no seasonality), adult female and immature fatalities are predictable in space, restricted to bisected riparian zones and roads close to intact forests. Adult male road fatalities can also be expected in seemingly unsuitable habitat areas. Our study shows how important correct interpretation of spatial, temporal and demographic data on road fatalities is and how experimental research prior to installing crossing structures could increase mitigation impact.

1. Introduction

Roads have substantial effects on the integrity of ecosystems worldwide as they cause habitat loss and fragmentation (Trobulak & Frissell 2000) and facilitate deforestation (Laurance et al. 2002). Road traffic directly threatens ecosystems by being a source of mortality to animals through vehicle collisions (commonly known as 'roadkill'; Forman & Alexander 1998, Clevenger et al. 2003) and as a barrier to animal movement (Shepard et al. 2008), decreasing gene flow between populations and increasing local extinction probability (Jackson & Fahrig 2011; Karlson et al. 2014). It is suggested that at some stage from the 1960's onwards vehicles probably overtook hunting as the leading direct human cause of terrestrial vertebrate mortality

(Forman & Alexander 1998). Future impacts will become even more prominent as the global number and extent of roads is anticipated to increase by at least 25 million km by 2050, a 60% rise in road length from 2010 (Laurance et al. 2014). Up to 90% of new road construction is expected in developing countries including areas sustaining exceptional biodiversity such as subtropical and tropical forests (Laurence et al. 2009; Dulac 2014; Laurance et al. 2017). Estimates show that of the world's remaining forest nearly 20% are now found within 100 m, 50% within 500 m and 70% within 1 km of an edge created through anthropogenic habitat fragmentation (Haddad et al. 2015).

Forests are home to a variety of arboreal animal taxa that depend on trees to varying degrees. Some species spend their entire life elevated in trees whereas others descend to the forest floor more regularly (Soanes & van der Ree 2015). Those who do come to the ground typically stay close to trees for safety and often show cautious behaviours such as running and displays associated with tension (Gregory et al. 2017). For most arboreal species a connected tree canopy is vital (Soanes & van der Ree 2015).

The majority of the worlds' primate species are arboreal, inhabiting forests; latest figures show that ~ 60% of primate species are threatened by extinction and that ~ 75% have declining populations due to ever increasing anthropogenic pressure (Estrada et al. 2017). Habitat loss and fragmentation due to the construction of linear infrastructure (roads and railways) impact up to 13% of primate species globally (Estrada et al. 2017). The lack of canopy connectivity can force arboreal species to leave trees in search of alternative routes to cross open areas, exposing them to risks such as vehicle collisions on roads. Records of affected primate species forming part of general vertebrate roadkill surveys are available (e.g. Drews 1995; Senzota 2012; Kioko et al. 2015) but research focusing specifically on primate road fatalities and mitigation is not extensive. Until recently, roadkill surveys were most common in developed countries (e.g. United States and Europe) (Collinson et al. in press) and although the first primate roadkill study was published in 1995 (Valladares-Padua) it took another 15 years for this issue to receive further attention. Still, less than a dozen studies from South America, Africa and Asia have explicitly researched primate roadkills (Valladares-Padua 1995; Mass et al. 2011; Teixera et al. 2013; Cibot et al. 2015; Donaldson & Cunneyworth 2015; Mclennan & Asiimwe 2016; Al-Razi et al. 2019) and of these only four (Valladares-Padua 1995; Mass et al. 2011; Teixera et al. 2013; Donaldson & Cunneyworth 2015;) included the use of mitigation measures.

Road crossing structures for wildlife encompass a broad range of underpasses and overpasses, all aimed at facilitating safe passage of animals (mitigating road fatalities) and ultimately conserving overall biodiversity (van der Grift & van der Ree 2015).

The most commonly used mitigation measures for arboreal, forest dwelling species are canopy overpasses/bridges. Designs of such canopy bridges range from simple rope ladder designs (Goosem et al. 2006; Weston et al. 2011) to hammock-like rope bridges (Goldingay et al. 2013;), rope tunnels (Goosem et al. 2006; Weston et al. 2011; Goldingay et al. 2013;) and vertical gliding posts (Taylor & Goldingay 2011; Goldingay et al. 2011), depending on target species. Designs used to date specifically for primate species include simple rope ladder bridges (used for vervet (*Chlorocebus pygerythrus*), Sykes' (*Cercopithecus mitis*) and Angolan colobus (*Colobus angolensis*) monkeys in Kenya (Donaldson & Cunneyworth 2015) and brown howler monkeys (*Alouatta guariba clamitans*) in Brazil (Teixeira et al. 2013)) and single, solid pole/pipe bridges (used for black lion tamarins (*Leontopithecus chrysopygus*) and tufted capuchins (*Cebus apella*) in Brazil (Valladares-Padua 1995) and for brown lemurs (*Eulemur fulvus*), red-bellied lemurs (*E. rubriventer*), greater dwarf lemurs (*Cheirogaleus major*), grey bamboo lemurs (*Hapalemur griseus*) and diademed sifaka (*Propithecus diadema*) in Madagascar (Mass et al. 2011)).

Many road ecology studies are non-experimental and lack a methodology which evaluates the use of crossing structures and optimises their design (van der Grift & van der Ree 2015; van der Ree et al. 2015), particularly regarding primate roadkill mitigation. Wild primates are frequently trained to get used to observers (a process called habituation) for behavioural studies (Williamson & Feistner 2003) which can offer unique opportunities for field experimentation aimed at directly informing conservation issues. Our study aims to fill gaps by using the South African samango monkey (*Cercopithecus albogularis*) as a model species to identify a suitable canopy bridge design for arboreal forest guenon roadkill mitigation. As with many other primate species, the samango monkey faces a direct mortality risk due to road infrastructure in its South African range (Linden et al. 2016). Our study includes two main elements: (1) recording and analysing area(s) where individuals are roadkilled and (2) testing bridge designs as a mitigation measure on habituated samango monkeys through field experimentation. The latter included testing the monkey's preference for two different canopy bridge designs and determining if degree of canopy cover over a road influences how they choose to cross (on the ground, tree or canopy bridge). We further recorded if the monkeys demonstrated behaviour such as increased vigilance or running when using our canopy bridge designs to make inferences on how cautious they were using an artificial structure. We make recommendations for conservation and management practitioners tasked with reducing primate road fatalities in particular and show how experimentation prior to installation may aid effectiveness of wildlife crossing structures in general.

2. Materials and Methods

2.1. Study species

The samango monkey belongs to the taxon-rich species group of guenons (Tribe Cercopithecini) which constitute half of the central African forest primate fauna (Grubb et al. 2003). Most guenons are highly arboreal forest dwellers, with three species (*Cercopithecus lhoesti, C. preussi, C. solatus*) considered semi-terrestrial (Butynski 2002). Guenons are widespread across and endemic to sub-Saharan Africa (41 countries) and the samango monkey represents the southernmost distribution of arboreal guenons (Ukisintambara & Thébaud 2002). Most guenons (including samangos) live in multi female, single adult male groups and males typically leave the maternal group when sexually mature. Extra group males often form small bachelor groups (Henzi & Lawes 1987; Butynski 1990; Cords 2001). There is much debate on the number of guenon genera, species and subspecies depending on species concepts used by taxonomists (Butynski 2002). The International Union for Conservation of Nature (IUCN) currently lists five genera, 27 species and 33 subspecies. Of those 27 species, one is listed as Critically Endangered, two as Endangered, seven as Vulnerable and one as Near Threatened (IUCN 2019). Of the 33 subspecies, one is listed as Critically Endangered, four as Endangered, seven as Vulnerable and two as Near Threatened (IUCN 2019). The samango monkey is globally listed as Least Concern by the IUCN, however in South Africa the subspecies of this study, *C. a. schwarzi*, is listed as Endangered and is of conservation concern (Linden et al. 2016).

2.2. Study area

Our study recorded and analysed samango road fatalities on a provincial paved road (R524) running south of and parallel to the Soutpansberg mountain range in northern South Africa (Limpopo Province; Figure 1). High canopy, evergreen forest habitat suitable for samango monkeys occurs only on the southern ridges of the mountain range. The R524 road between the towns of Louis Trichardt and Thohoyandou is a single lane road (approximately 80 km in length) that intermittently widens on either side to allow for overtaking. The width of the road is ~ 6 m for double and ~ 9 m for triple lane sections. The speed limit along this road is 100 km/h. This road runs close to a sizable indigenous forest patch in one area and bisects riverine forests in another. However, canopy cover is never closed over the road as verges have been generously cleared of vegetation (Figure 2).

Experimental work was conducted at the Lajuma Research Centre, situated in the far west of the Soutpansberg (Figure 1) where two well-habituated groups of samango monkeys inhabit a patch of Limpopo Mistbelt Forest (Geldenhuys & Mucina 2006). The road infrastructure at the research centre consists of approximately two and half metre-wide unpaved roads bisecting high canopy forest and woodland. For our experiment we focussed on one of the habituated samango groups, comprising ~ 60 individuals including a single breeding adult male, adult females, subadults, juveniles and infants. Extra group adult males were also recorded frequently during data collection. The group was known to cross the farm roads daily and GPS localities for the most frequented crossing points were available.

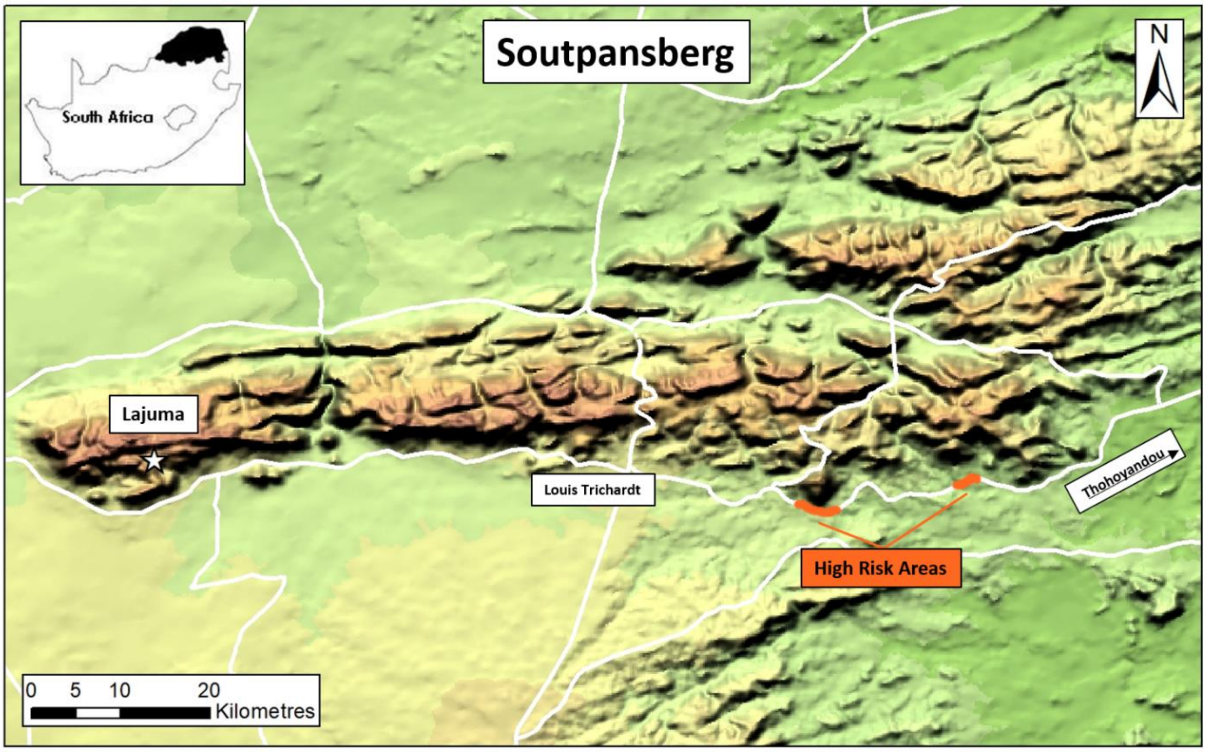

Figure 1. Location of the two Soutpansberg study areas indicating national and provincial road networks (white lines) in Limpopo Province, South Africa. Experimental work was undertaken at the Lajuma Research Centre and data on road fatalities (High Risk Areas) was collected on the provincial paved R524 road between the towns of Louis Trichardt and Thohoyandou. Map data: Chief Directorate: National Geo-spatial Information, Republic of South Africa.

2.3. Roadkill data

Through articles placed in two local newspapers, members of the public were asked to report samango monkey roadkill incidents on roads in the Soutpansberg area. People were requested to take a GPS point or Google Earth placemark of the roadkill(s) and contact us to verify the report and remove each samango monkey carcass. Further, the road we focused on in this study is driven by ourselves and colleagues several days a week commuting to work. Data on roadkilled individuals were collected over a seven-year period (from 2012 – 2019). For each individual observed, the GPS coordinates and age-sex class were recorded. The latter included adult male (M), adult female (F), subadult female (SAF), subadult male (SAM), juvenile female (JF), juvenile male (JM), infant female (IF) and infant male (IM). Location data was analysed using 2008 aerial photography (Chief Directorate: National Geo-spatial Information, Republic of South Africa) in ArcGIS 10.5 (Esri®) to assess roadkill site specific characteristics including land use matrix on either side of the road and canopy cover over the road. Locations of all road fatalities were used to demarcate high risk zones (areas where roadkills are concentrated) for samango monkeys and to identify possible sites for future canopy bridge installation.

2.4. Bridge experiment

Samango monkey's preferences were explored by offering the two canopy bridge designs so far used as primate roadkill mitigation measures: the flexible rope bridge and the single pole bridge design. We used rigid bamboo trunks for the single pole bridge design (~ 5 m in length). Ladder rope bridges were constructed using two parallel nylon ropes (12 mm diameter), with 30 cm long wood cross-pieces spaced 30 cm apart along the ropes. We targeted 25 known points on Lajuma Research Centre where the group frequently crossed the unpaved roads. Treatments were paired with one pole bridge and one rope bridge at each of the 25 sites. Bridges were fixed to trees on each side of the road. The height of the bridges across the road varied between 3 – 4.5 m depending on the canopy height on each side of the road.

To assess if canopy cover across the road had an effect on how the monkeys crossed the road (using either the bridge, tree canopy, or ground) we classified three canopy cover scenarios for the 25 localities: 'open' (no canopy cover), 'partial' (some canopy cover but not continuous across the road), 'closed' (canopy cover continuous across the road) resulting in nine 'open', nine 'partial' and seven 'covered' pairs. As the extent of canopy cover across the road was varied, little additional manual clearing for the 'open' canopy category was required.

2.5. Behavioural data

Behavioural data on monkey road crossings was collected at the Lajuma Research Centre over eight months (April - December 2016). We evaluated samango monkey behaviour through direct observation of each road crossing event. A crossing event was defined as an individual crossing the road by bridge, in the canopy or on the ground within five m of a bridge. Data were collected every time the monkeys crossed the road which was typically twice a day, between 6.00 am – 8.30 am and between 2.00 pm – 6.00 pm. We observed a total of 2119 crossing events over 100 observation days, averaging three observation days per week.

For each event the date, time taken to cross, age-sex class and number of individuals crossing by canopy, bridge or on the ground was recorded. For each individual crossing on a bridge, the design used (rope or pole) was noted. Age-sex classes were adult female (F), adult male (M), subadult (SA), juvenile (J) and infant (I) and as infants are mostly carried by their mothers we included the category female with infant (FI). Sex differentiation of immature individuals (SA, J, I) was not possible due to absence of secondary sexual characteristics.

Vigilance and crossing behaviour were used as measures of how cautious the monkeys behaved when using bridges under different canopy cover scenarios. For the latter we noted two crossing behaviours: ‘running’ indicating cautious behaviour and ‘sitting’ indicating that the animal was comfortable on the structure.

Vigilance was quantified as the number of glances while crossing the bridge. Number of glances as a measure of vigilance has been used for many primate behavioural studies (e.g. Treves 2000; Gaynor & Cords 2012). We defined glance as an event during which the monkey scanned its environment beyond its immediate vicinity, distinguished from foraging behaviour (individual is looking for food) and from locomotion behaviour (individual is looking to find its way). The number of neighbours within a radius of five m around the focal individual was also noted as this can influence vigilance levels (Allan & Hill 2018). Weather was recorded as an environmental variable and was categorised as ‘clear’, ‘cloudy’ or ‘rain’ and wind was classified as ‘calm’, ‘breeze’ or ‘windy’. We included these as we suspected that windy and/or rainy conditions might influence vigilance levels as they could obscure an animals visual and auditory perceptions.

After completing behavioural observations, we undertook an arboreal camera trap survey at 20 bridge crossing sites to monitor the use of the bridges by animals other than samango monkeys (Gregory et al. 2014). Ten camera traps (Ltl Acorn 6210MC) were mounted in pairs and rotated equally between the 20 sites for six weeks yielding 24 survey days/nights for each bridge site.

2.6. Statistical analysis: bridge experiment and behavioural data

Statistical analyses were done using R version 3.6.1 (R Core Team 2019). To test whether monkeys preferred a specific bridge design (rope vs. pole) we used a Wilcoxon signed-rank test to assess differences in the number of records between the two bridge designs within bridge pairs.

For the behavioural data we used general linear mixed models (GLMM) and fitted them using Bayesian statistics with Markov Chain Monte Carlo (MCMC) simulations to assess the effect of age-sex class and canopy cover on the crossing way (ground, tree canopy, bridge) (multi-response models 1 and 2) (Hadfield 2010), on vigilance (model 3) and on the probability of monkeys to sit and run on the bridge (models 4 and 5). For all models bridge ID, name of observer and date were included as random effects. We used the MCMCglmm R package (Hadfield 2010) and made our inferences using 95% High Posterior Density (HPD) credibility intervals (CrI) of the parameter estimates for fixed effects and random effects. Significance of the differences between fixed effects was assessed according to the overlap of 95% CrI. To ensure convergence and adequate chain mixing of each model we examined the trace plots, effective sample sizes (ensuring it approached 1 000 per run) and posterior distribution densities (Hadfield 2010; Wilson et al. 2010; de Villemereuil 2012). We used weakly informative priors for all five models (V = 1, nu = 0.002). Table 1 provides specifications for each of the five models. Models 1 and 2 are multi-response GLMM's allowing analysis of several response variables simultaneously (Hadfield 2010). For the vigilance model (model 3) we included time taken to cross the bridge as a fixed effect to correct the number of glances according to the duration of the crossing event.

Table 1. Specifications for each of the five model (MCMCglmm) runs for behavioural analysis of samango monkey crossing events with the model number, behaviour, response variable(s), fixed effects, family, iterations, burn-in period and thinning interval.

Model	Behaviour	Response	Fixed effects	Family	Iterations	Burn-in	Thinning
1	Crossing way	Occurrence of crossings by tree, bridge, ground	~way+way:cover	binomial	130 000	300 000	1 000
2	Crossing way	Occurrence of crossings by tree, bridge, ground	~way+way:sex	binomial	130 000	300 000	1 000
3	Vigilance	Number of glances	~time+number of neighbours+weather conditions+windy+ age-sex:canopy cover	poisson	2.6 mio	600 000	2 000
4	Sit	Occurrence of sitting events on bridge	~age-sex+canopy cover+age-sex:canopy cover	binomial	260 000	60 000	200
5	Run	Occurrence of running events on bridge	~age-sex+canopy cover+age-sex:canopy cover	binomial	1.95 mio	450 000	1 500

3. Results

3.1. Roadkills

We recorded 20 samango monkey roadkills and two orphaned infants over a seven-year period (Table 2) on the R524 road. Roadkilled individuals represented all age-sex classes with 14 adults (n = 8 M, n = 6 F) and six immature individuals, including the two orphans (n = 2 MI, n = 1 JF female, n = 2 SAF, n= 1 SAM). Roadkills occurred throughout the year (10 out of 12 months) during both the dry, May - September (n = 10) and wet, October - April (n = 10) seasons. Most samango monkey roadkills were recorded in 2013 (n = 6) and 2015 (n = 6).

The matrix on either side of the road in roadkill localities was either indigenous, largely riparian forest (natural vegetation), fruit orchards or *Eucalyptus* spp. plantations (exotic monocultures). Only adult male roadkills were found in localities where both sides of the road comprised exotic monocultures (Table 2). Female and immature road fatalities always occurred in areas where at least one side of the road comprised indigenous forest. The canopy cover above the road was always open except for one locality where the canopies of large trees on either side of the road covered the verges partially. Our results show two particular road stretches, one less than 500 m just south of a sizeable indigenous forest patch and one bisecting riparian forest, where samango monkey roadkills were concentrated (Figure 2 a and b). In each of these two high risk areas we found several sites potentially suitable for canopy bridge installation either because of high roadkill densities or because of suitable vegetation along verges (Figure 2 a1, a2, b1 and b2).

Table 2. Roadkilled samango monkeys collected on the R524 road between 2012 – 2019 in the Soutpansberg detailing the month, season, age-sex class, matrix (indigenous forest ("forest") or exotic monocultures) on both sides (north, south) of the road and canopy cover above the road. Age-sex classes include adult male (M), adult female (F), subadult female (SAF), subadult male (SAM), juvenile male (JM), juvenile female (JF) and infant male (IM).

Year	**Month**	**Season**	**Age-sex**	**Matrix**	**Canopy cover above road**
2012	June	Dry	M	Fruit orchard	Open
2013	February	Wet	F	Forest	Open
2013	April	Wet	M	Eucalyptus plantation, forest	Partial
2013	April	Wet	M	Fruit orchard	Open
2013	August	Dry	SAF	Fruit orchard, forest	Open
2013	October	Wet	M	Eucalyptus plantation	Open
2013	November	Wet	SAM	Eucalyptus plantation, forest	Open
2014	September	Dry	M	Fruit orchard, forest	Open
2014	December	Wet	F, JF	Forest	Open
2015	June	Dry	2 F, 2 JM	Forest	Open
2015	July	Dry	SAF	Fruit orchard, forest	Open
2015	November	Wet	F (IM*)	Forest, fruit orchard	Open
2016	June	Dry	M	Fruit orchard, forest	Open
2017	February	Wet	F (IM*)	Fruit orchard, forest	Open
2018	May	Dry	M	Forest	Open
2019	February	Wet	M	Fruit orchard, forest	Open

* orphaned infant

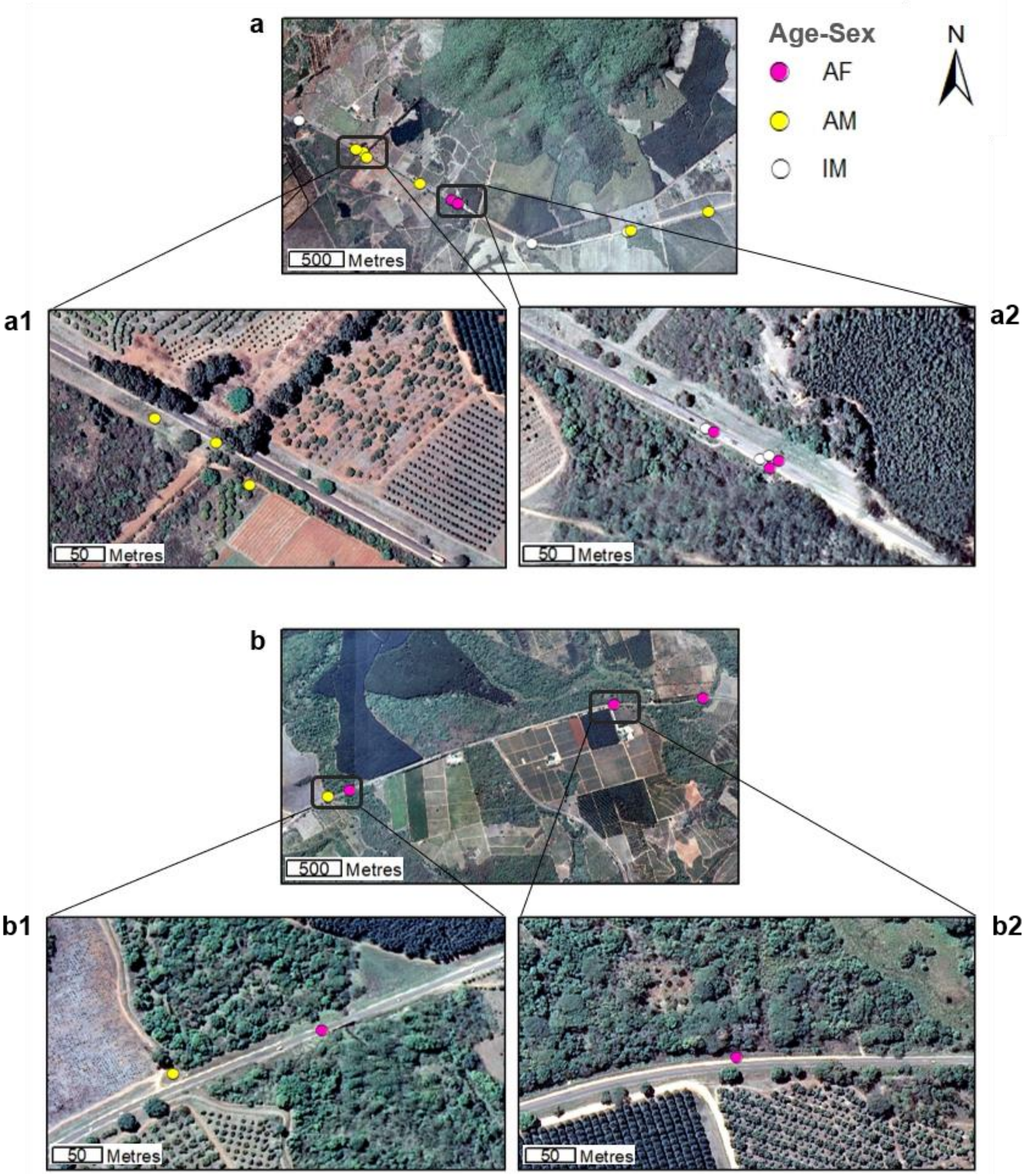

Figure 2. Localities of the two high risk roadkill areas (as shown in Figure 1) along the provincial paved road (R524). Area a shows the stretch of paved road just south of a sizable indigenous forest patch. Enlargements show areas of interest for canopy bridge installation. Enlargement a1 shows a site with a high concentration of adult male roadkills and enlargement a2 shows an area with a high number of adult female and immature roadkills. Area b shows the stretch where the paved road bisects riparian forest. Enlargement b1 and b2 show roadkills (adult male and adult female) in that area. Aerial imagery: Chief Directorate: National Geo-spatial Information, Republic of South Africa.

3.2. Bridge design

Significant differences were found in the number of crossing events between the two types of bridges. Samango monkeys clearly preferred the single pole design over the rope design when crossing the road on a canopy bridge (Wilcoxon signed-ranked test, n = 25 (number of bridge pairs), V = 325, p-value < 0.001) (Figures 3 and 4). As rope bridges were used at extremely low frequency they were excluded from any further analysis.

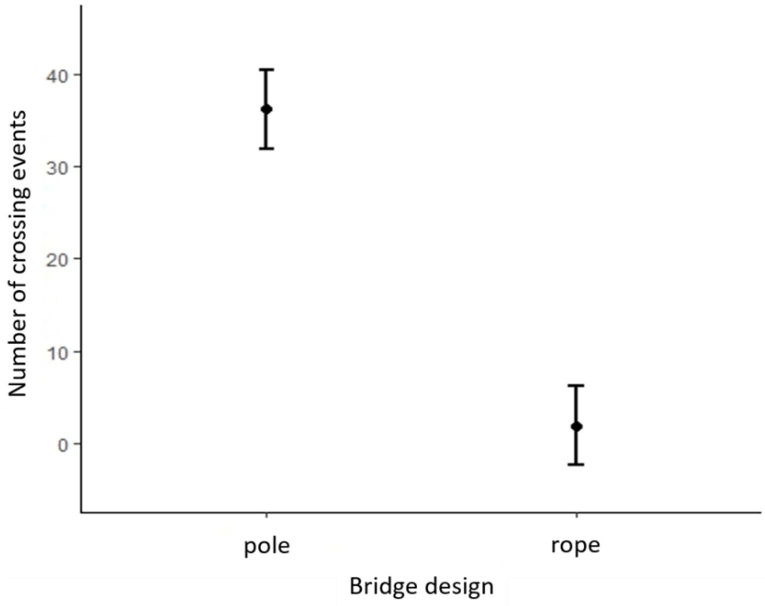

Figure 3. Number of samango crossing events recorded for both bridge designs at the Lajuma Research Centre (normed mean ± se). Variations between individuals (i.e. bridge pairs) were removed according to the methods of Cousineau (2005) and the correction of Morey (2008).

Figure 4. Samango monkeys using the two bridge designs (a, b) and choosing between them (c) to cross a road at the Lajuma Research Centre (photographs: Q. Horta-Lacueva). Note how the monkey does not use the wooden cross-pieces of the rope bridge but rather balances using the two parallel ropes (a).

3.3. Effect of canopy cover and age-sex class on the road crossing mode

We investigated if the three canopy cover scenarios and age-sex classes affected how the monkeys crossed the road (using either ground, trees or the bridge) (Figure 5). Canopy cover had an effect on how the monkeys chose to cross the roads (Table 3, model 1 and Figure S.1). Sharp differences between means of the posterior estimates showed that the monkeys preferred using the bridges rather than crossing on the ground, while the choice between using the trees and the bridges was largely a function of the degree of canopy cover. Our confidence in these patterns is supported by the weak overlaps between the 95% credible intervals (95% CrI) of those estimates. In the same model, the mean of the posterior estimates related to using the ground under open canopy was lower than the baseline, which indicates that the monkeys preferred bridges to the ground when canopies were open, although the 95% CrI overlapped with 0. This effect might also be related to the fact that monkeys generally avoided crossing in areas with open canopy, however if they did cross in these areas, there was an overwhelming preference for using the bridges (Figure 5).

The crossing way was also influenced by the age-sex class (Table 3, model 2 and Figure S.2). According to the mean of posteriors estimates and the non-overlapping credible intervals, juveniles showed a lower propensity to cross on the ground than adult females. Adult males also tended to show a higher propensity to cross on the ground instead of on the bridge compared to females, although their 95% CrI slightly overlap. Males, juveniles and subadults showed a higher propensity to use trees instead of bridges compared to females, although the three former age-sex classes did not differ from one-another (the mean of the posteriors density of the juveniles is lower than in the adult males and the subadults but the 95% CrIs of the three groups widely overlap).

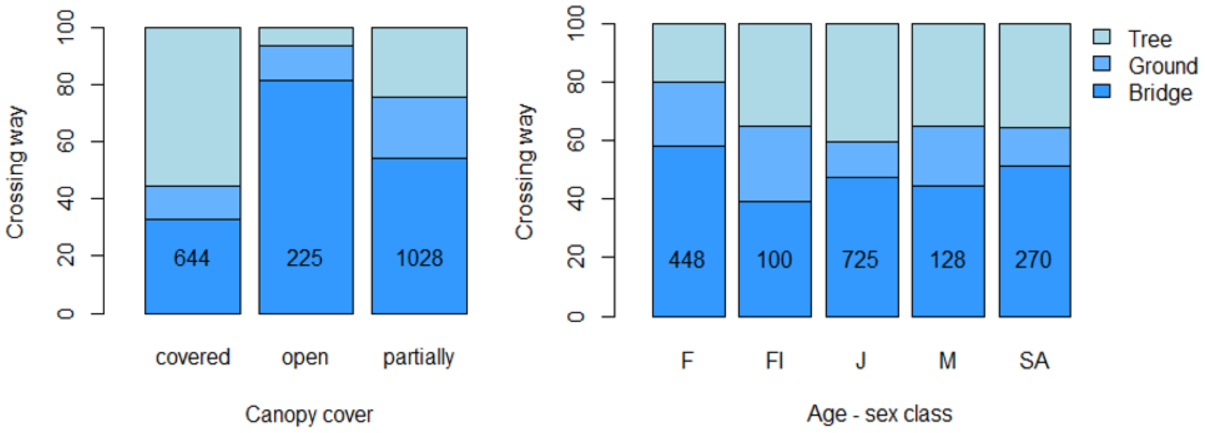

Figure 5. Proportion of crossings by tree, ground and bridge (crossing way) when considering canopy cover (covered, open, partially covered) (left) and age-sex class (F = adult female, FI = female carrying infant, J = juvenile, M = adult male and SA = subadult) (right). Values in bars indicate the total number of observations.

Table 3. Fixed effects (posterior mean shown) and 95% HPD credible intervals (95% CrI) (lower (l-) and upper (u-) CrI) for the of the two multi-response models investigating the effect of canopy cover (model 1) and age-sex (model 2) on the road crossing way (bridge, tree, ground). The baseline of both models was female + bridge.

	mean	**l-CrI**	**u-CrI**
crossing model 1			
response(way.ground)	-1.71	-3.35	-0.19
response(way.tree)	0.17	-1.25	1.76
response(way.ground):coveropen	-1.57	-3.17	0.21
response(way.tree):coveropen	-3.96	-5.61	-2.11
response(way.ground):coverpartially	-0.21	-1.5	1.53
response(way.tree):coverpartially	-1.93	-3.21	-0.25
crossing model 2			
response(way.ground)	-2.16	-3.42	-0.8
response(way.tree)	-2.25	-3.55	-0.92
response(way.ground):sexFI	0.16	-0.6	0.84
response(way.tree):sexFI	0.59	-0.08	1.3
response(way.ground):sexJ	-0.77	-1.18	-0.34
response(way.tree):sexJ	0.68	0.29	1.05
response(way.ground):sexM	0.51	-0.18	1.14
response(way.tree):sexM	1.2	0.61	1.80
response(way.ground):sexSA	-0.28	-0.80	0.26
response(way.tree):sexSA	0.91	0.41	1.36

3.4. Effect of canopy cover and age-sex class on vigilance and the probability of sitting or running when using a canopy bridge

Analysis of vigilance levels showed that age-sex class and canopy cover did not appear to affect the number of glances overall (Table S.1, model 3). The monkeys' probability to sit on a bridge when crossing a road was also not affected by either canopy cover or age-sex class (Table S.1, model 4). The behaviour run was found to be affected by canopy cover and age-sex class. There was strong support for the monkeys to run more under open and partial canopy cover compared to closed canopy. Juveniles tended to run more when crossing bridges compared to the other age-sex classes, although large overlaps in the credible intervals indicates that this pattern should be interpreted with caution (Table 4, model 5 and Figure S.3).

Table 4. Fixed effects (posterior mean shown) and 95% HPD credible intervals (95% CrI) (lower (l-) and upper (u-) CrI) for the model investigating the effect of age-sex and canopy cover on the bridge crossing behaviour "run" (model 5).

	mean	**l-CrI**	**u-CrI**
run model 5			
covercovered (Intercept)	-4.16	-6.17	-2.5
coveropen	2.03	-0.14	4.08
coverpartially	1.52	-0.13	3.33
sexFI	1.71	-0.93	4.3
sexJ	3.59	1.8	5.45
sexM	1.84	-0.58	4.67
sexSA	2.19	0.11	4.53
coveropen:sexFI	-451.73	-638.42	-289.17
coverpartially:sexFI	-0.56	-3.55	2.3
coveropen:sexJ	-0.68	-3.23	1.49
coverpartially:sexJ	-0.3	-2.3	1.64
coveropen:sexM	-1.83	-6.34	2.01
coverpartially:sexM	-1.87	-5.35	1.27
coveropen:sexSA	-1.46	-4.35	1.36
coverpartially:sexSA	-0.31	-2.6	2.14

3.5. Bridge use by other species

In total, 26 956 images were taken during the camera trap survey yielding 911 individual crossing events. In addition to the samango monkey, we identified another four mammals to species and one to order level (Hyracoidea) from camera trap images. Rodents and reptiles captured on camera could not be identified to species level from the images (Figure 6, Figure S.4). Across all animal taxa recorded crossing on bridges the single pole bridge was clearly preferred over the rope ladder bridge (94%, n = 856 on pole bridge, 6%, n = 55 on rope bridge). Samango monkeys were by far the most frequently recorded animals crossing on the bridges (80%, n = 726) followed by the two nocturnal primate species *Otolemur crassicaudatus* (9%, n = 81) and *Galago moholi* (6%, n = 55) (Figure 6). We recorded a gecko once on a pole bridge but did not include this as a crossing event. Three raptor species were recorded perching on the pole bridges, namely African goshawk (*Accipiter tachiro*), African cuckoo-hawk (*Aviceda cuculoides*) and African wood owl (*Strix woodfordii*).

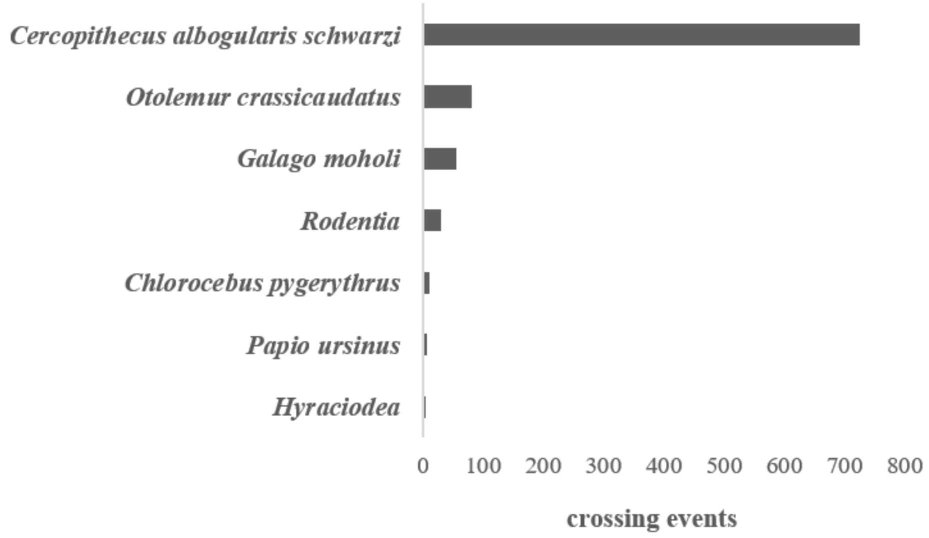

Figure 6. Total number of bridge crossing events recorded through camera trapping for each animal taxon.

4. Discussion

4.1. Use and design of canopy bridges

Our experimental results show that canopy overpasses are a viable intervention for bridging gaps in the tree canopy to mitigate arboreal guenon road fatalities. We found that if canopy bridges are present under open and partial canopy cover scenarios, the probability of samango monkeys crossing on the ground, and with that the risk of car collisions, can be reduced.

Although there were differences between how age-sex classes chose to cross the road (e.g. using the ground, canopy, and/or bridge) we can demonstrate that overall, all age-sex classes accepted the canopy bridges as a means of crossing a road. When natural tree canopy was not available for road crossings, all age- sex classes preferred to cross the road on a bridge rather than on the ground.

As would be expected from an arboreal forest dweller, our results clearly showed that the monkeys preferred to cross the roads using trees if closed canopy cover was available. This suggests that the most suitable and cost-effective solution for mitigating arboreal guenon road fatalities would be to retain natural canopy cover at specific spots during road construction. Gregory et al. (2017) found that the benefit of natural canopy bridges is that they require very little habituation for arboreal species and that, if left in places of previously known animal paths, they can result in rapid mitigation. However, leaving natural canopy crossings during road construction is probably not always feasible for logistical (e.g. very wide roads) and road safety reasons (falling trees or branches) and in cases like ours where trees have been cleared leaving no connected canopy across an existing road, artificial crossing structures for mitigation are the only option for offering a safer alternative to cross a road.

While the probability of the monkeys to sit on the bridges was not affected by canopy cover and age-sex class, the monkeys' showed an increased probability to run across bridges under open and partial treatments compared to closed canopy, indicating that they were more cautious when exposed. Aerial predators such as crowned eagles (*Stephanoaetus coronatus*) are one of the main predators of forest guenons. Cords (1990) found in blue monkeys (*C. mitis*) that the rate of looking up decreased with increased foliage and linked that to vigilance for aerial predators. When looking at the behaviour run, juveniles tended to show the highest probability to do so indicating increased caution when crossing on a canopy bridge. Results from our vigilance analysis did not allow us to draw any conclusions. Primate vigilance behaviour is intrinsically complex, and studies often produce contradictory results (Allan & Hill 2018). We suggest that cautious behaviours such as running might be more informative for gauging how comfortable primates are when crossing an artificial canopy bridge than using glance rates. Future experiments comparing pole bridges with and without artificial covers could help to establish if these would reduce cautious behaviours and increase the monkeys' comfort when using an artificial canopy bridge.

Regarding the most suitable design of canopy bridges for arboreal guenons the samango monkeys showed a clear preference for single pole bridges over the rope ladder bridges. Being adapted to move along tree branches, this design seems to accommodate their natural method of locomotion. Our camera trap survey revealed that other mammal species present in the study area also preferred the single pole bridge. We found that all five primate species (samango and vervet monkeys, greater and lesser bush babies and baboons) occurring in South Africa could potentially benefit from single pole canopy bridges with vervet monkey and greater bush baby road fatalities being commonly observed in the study area (Linden pers. obs.). Although Donaldson & Cunneyworth (2015) suggested that larger, terrestrial primate species such as baboons will not use canopy bridges, we found from camera trap images that subadult baboons did use pole bridges. Furthermore, the single pole bridge design has shown to be an effective roadkill mitigation measure for some South American (Valladares-Padua 1995) and Madagascan (Mass et al. 2011) primate species and our study shows it is a promising design not only for Africa's diverse forest guenon group but also for a variety of other primate and non-primate taxa.

4.2. Application and recommendations

Our roadkill data revealed that there was neither a seasonal pattern of road fatalities nor a difference between age-sex classes recorded. Despite the lack of evidence for differences amongst age-sex classes in road crossing behaviour, our data showed that on a landscape scale, male samangos are more at risk from road collisions in localities that could otherwise be considered low risk areas for forest guenons (both sides of the road bordering exotic monocultures). Linden et al. (in prep.) found that extra group males are regularly found in the matrix surrounding natural forest patches in the study area whereas maternal groups were only recorded in or close to their natural forest habitat.

From a population conservation perspective, extra group males are as important as maternal groups since they ensure gene flow between and thus maintenance of genetic diversity in local populations. Canopy bridges installed only in areas where maternal groups are known to cross roads would therefore not necessarily ensure genetic connectivity.

We collected a total of 20 roadkilled samango monkeys over a seven-year period (Mean = 2.9 individuals/year), leaving two juveniles orphaned. Whilst this number might appear low in comparison to other studies (e.g. Donaldson & Cunneyworth 2015), simple counts of wildlife corpses along roads can severely underestimate the actual mortality rate which can be up to 12-16 times higher than numbers detected (Slater 2002). For example, incidences of animals injured and dying some distance away from the road due to vehicle collisions are almost impossible to detect (Guinard et al. 2012) and consequently, the true number of actual roadkill depends on factors including presence of scavenger species, type and topography of road, time of day, road traffic and weather (Slater 2002). Taking this into account, we estimate that local samango monkey roadkill rates may be significantly higher than our data suggest, and local populations could suffer significant reductions over time if mitigation measures are not adopted as per our recommendations.

Our study of samango monkey roadkills in South Africa shows how important correct interpretation of spatial, temporal and demographic data are for road fatalities, as well as experimental research as a tool to understanding appropriate mitigation measures prior to installing correctly designed crossing structures. As van der Grift et al. (2013) state "*without well performed evaluations of the effectiveness of road mitigation measures, we may endanger the viability of wildlife populations and waste financial resources by installing structures that are not as effective as we think they are*".

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Supplementary

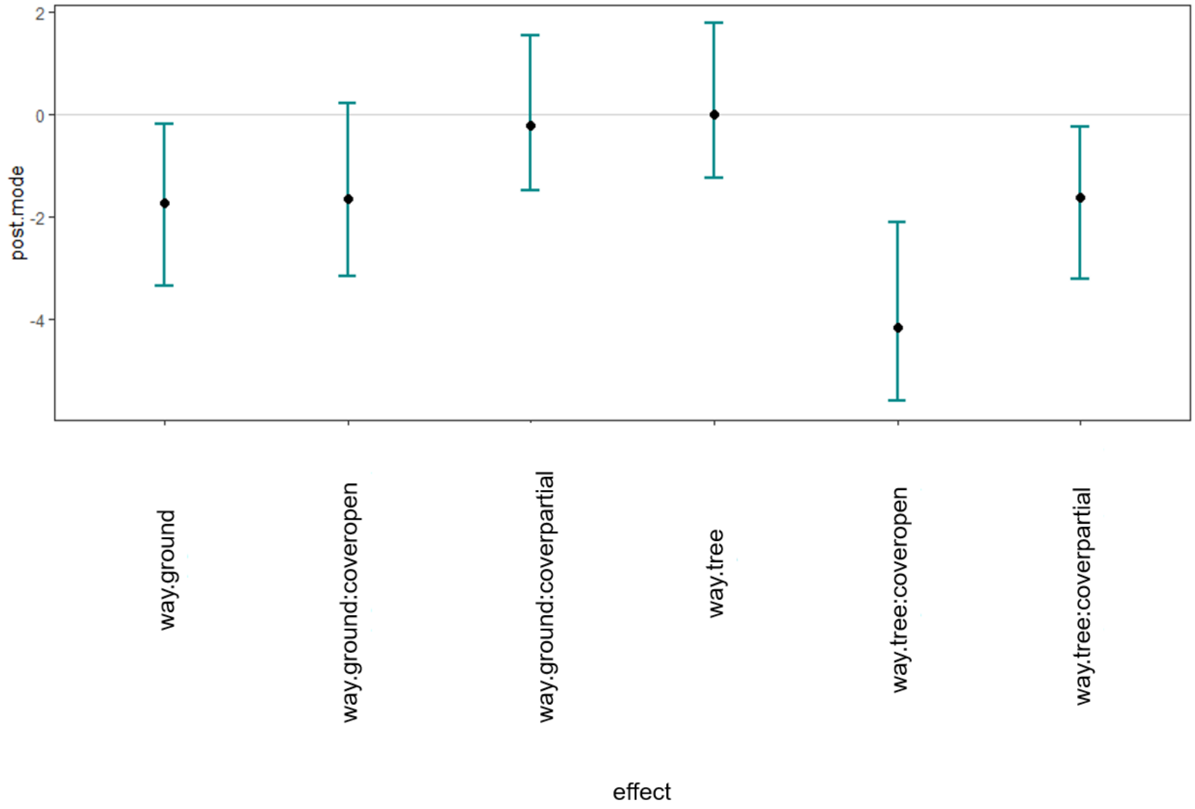

Figure S.1. Posterior modes and 95% HPD credible intervals (95% CrI) of the fixed effects (canopy cover) on the crossing way (ground, tree, bridge) of samango monkeys when crossing a road. Baseline: Adult females using bridge.

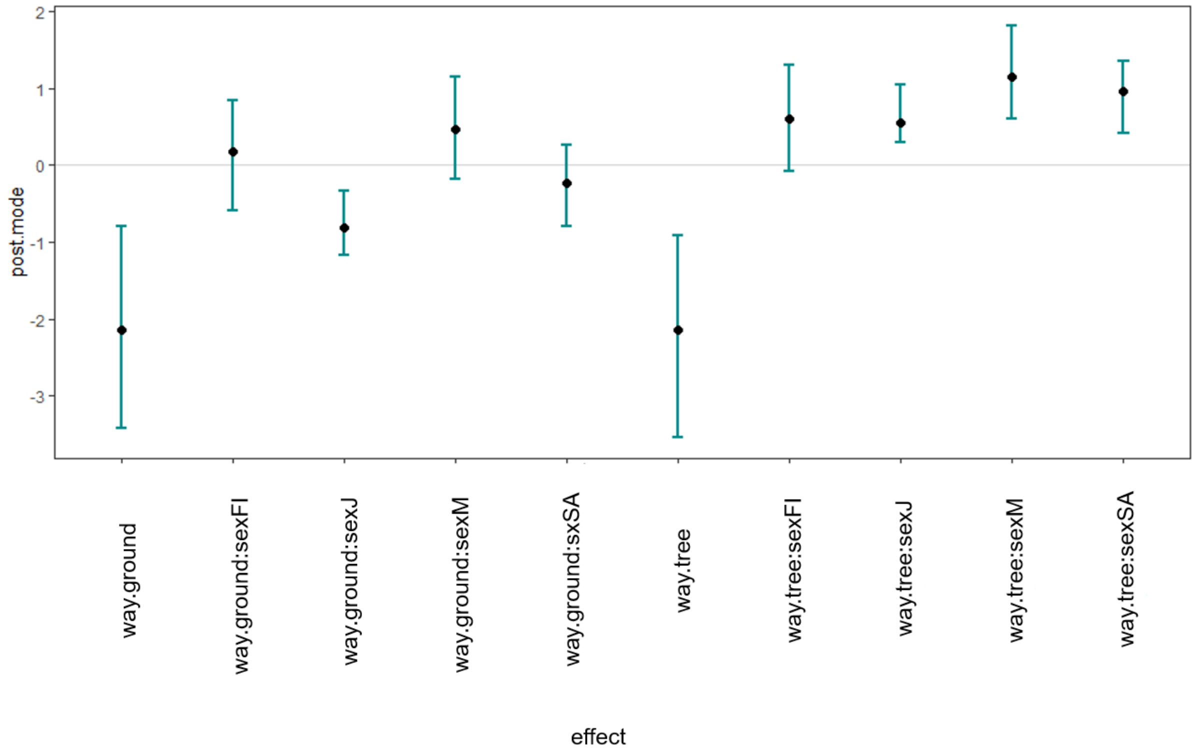

Figure S.2. Posterior modes and 95% HPD credible intervals (95% CrI) of the fixed effects (age-sex class) on the crossing way (ground, tree, bridge) of samango monkeys when crossing a road. Baseline: Adult females using bridge. Age-sex classes: M = adult male, FI = female with infant, SA = subadult, J = juvenile.

Table S.1. Fixed effects (posterior mean shown) and 95% HPD credible intervals (95% CrI) (lower (l-) and upper (u-) CrI) for the models investigating the effect of age-sex class and canopy cover on vigilance (model 3) and the bridge crossing behaviour sit (model 4).

	mean	**l-BCI**	**u-BCI**
vigilance model 3			
(Intercept)	0.36	-0.18	0.99
time	0.00	0.00	0.01
neighbours	-0.04	-0.11	0.03
weathercloudy	-0.13	-0.32	0.09
weatherrainy	-0.67	-1.8	0.42
windycalm	-0.00	-0.22	0.2
windywindy	0.32	-0.07	0.82
sexF:covercovered	-0.36	-0.73	0.037
sexFI:covercovered	-0.31	-0.91	0.23
sexJ:covercovered	0.18	-0.2	0.52
sexM:covercovered	-0.34	-1.03	0.25
sexSA:covercovered	0.07	-0.4	0.5
sexF:coveropen	0.2	-0.2	0.59
sexFI:coveropen	-0.12	-0.81	0.55
sexJ:coveropen	-0.24	-0.64	0.16
sexM:coveropen	-0.72	-1.5	0.12
sexSA:coveropen	0.18	-0.23	0.62
sexF:coverpartially	0.01	-0.26	0.28
sexFI:coverpartially	-0.1	-0.59	0.36
sexJ:coverpartially	0.04	-0.21	0.32
sexM:coverpartially	-0.32	-0.8	0.193
sit model 4			
covercovered (Intercept)	-1.61	-3.167	-0.23
coveropen	0.31	-1.66	2.3
coverpartially	0.14	-1.64	1.76
sexF	0.95	-0.62	2.46
sexFI	0.017	-2.32	2.15

sexJ	0.79	-0.71	2.31
sexM	1.12	-0.77	2.87
coveropen:sexF	-0.04	-2.07	2.3
coverpartially:sexF	-0.08	-2.1	1.52
coveropen:sexFI	0.84	-2.71	4.29
coverpartially:sexFI	1.03	-1.75	3.56
coveropen:sexJ	-0.96	-3.1	1.28
coverpartially:sexJ	-0.46	-2.22	1.3
coveropen:sexM	0.61	-2.32	3.35
coverpartially:sexM	-0.98	-3.33	1.28

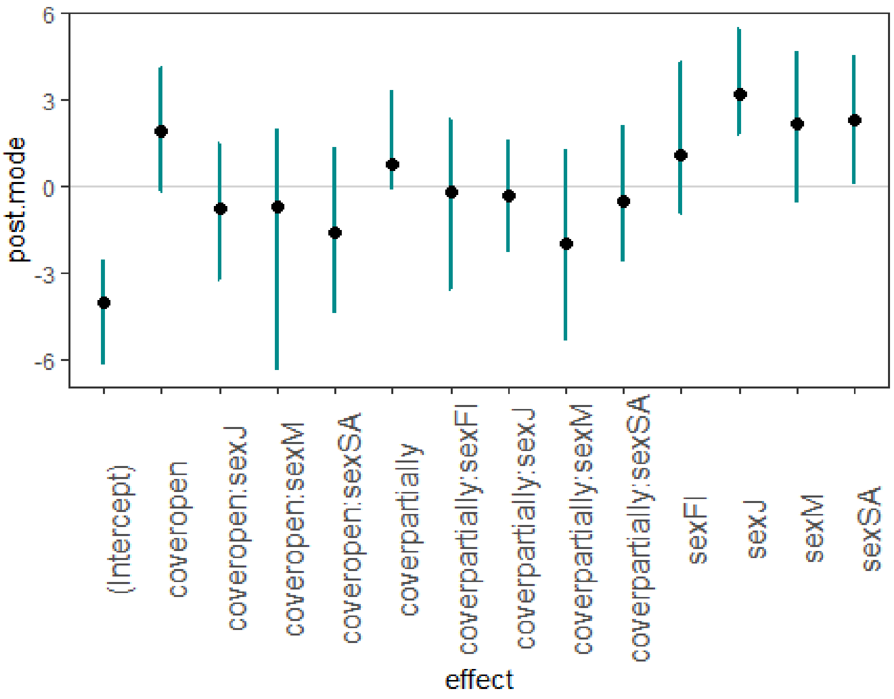

Figure S.3. Posterior modes and 95% HPD credible intervals (95% CrI) of the fixed effects (canopy cover and age-sex class) on the probability of samango monkeys to run when crossing a road on a canopy bridge. Intercept: covercovered. Age-sex classes: M = adult male, FI = female with infant, SA = subadult, J = juvenile.

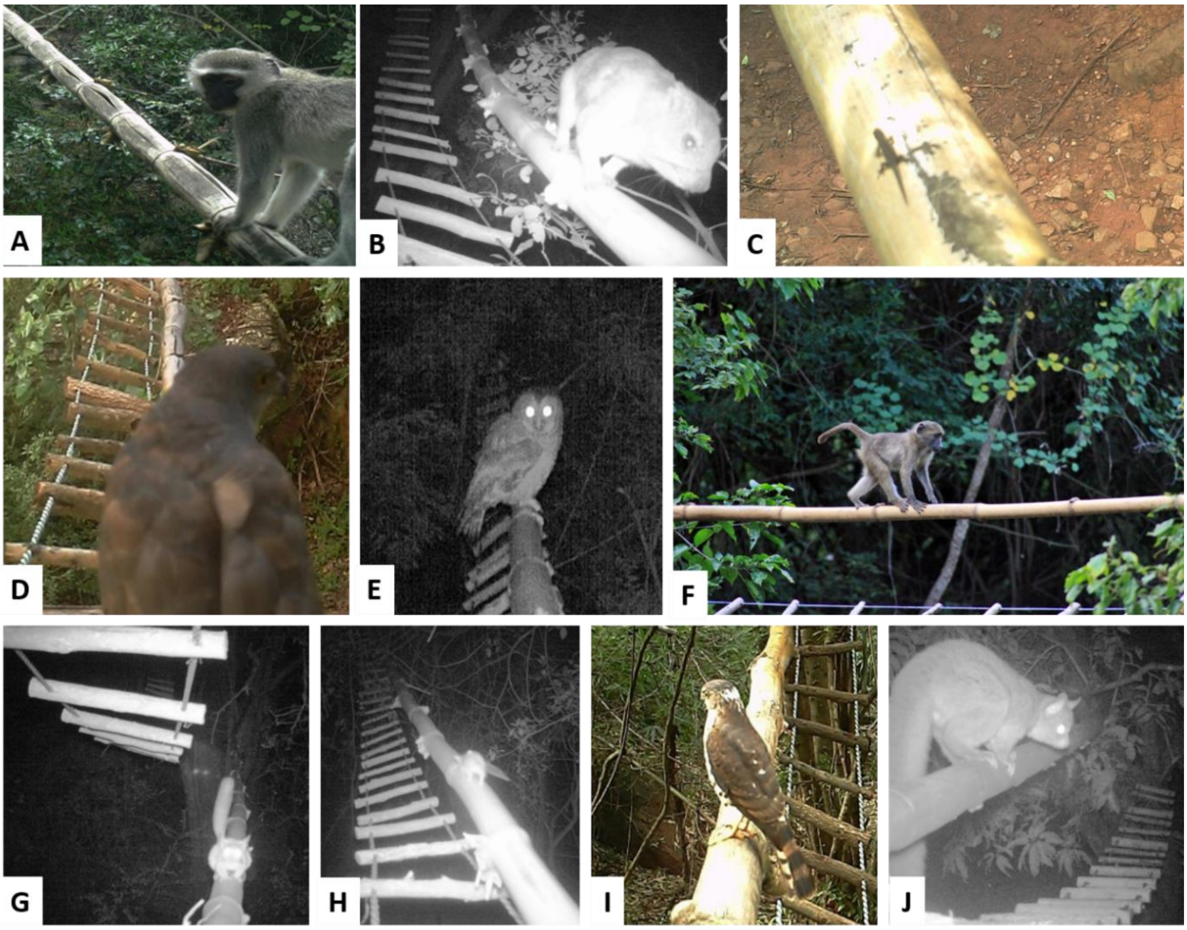

Figure S.4. Records of other animals using the canopy bridges. A) vervet monkey (*Chlororcebus pygerythrus*), B) hyrax (Hyracoidea), C) reptile (gecko), D) African goshawk (*Accipiter tachiro*), E) African wood owl (*Strix woodfordii*), F) chacma baboon (*Papio ursinus*), G) lesser bush baby (*Galago moholi*), H) rodent, I) African cuckoo-hawk (*Aviceda cuculoides*) And J) greater bush baby (*Otolemur crassicaudatus*).

Chapter 5: Adding another piece to the southern African *Cercopithecus* monkey phylogeography puzzle

Abstract

The taxonomy and number of *Cercopithecus* monkey radiation events in southern Africa are still debated. Genetic studies to date have largely been limited to single specimens per taxon and scattered geographical distribution. A recent study focussing on South African *Cercopithecus* monkeys showed that populations can be divided into three distinct genetic entities. Our current study aims to add new mtDNA and microsatellites data from a coastal population (Vamizi Island) in Mozambique to compare to existing data from South Africa. Our additional data allow a further test of the number and timing of radiation events of *Cercopithecus* monkeys in southern Africa. Here we propose the occurrence of a single, north-south radiation event during the mid-Pleistocene along the Afromontane forest belt and that after the Last Glacial Maximum, samango populations re-radiated into (re)established coastal forests on a more local scale. Our population genetic data support this pattern for both Mozambican as well as South African samango monkey populations.

1. Introduction

The taxonomy of *Cercopithecus* monkeys in southern Africa (commonly referred to as samango monkeys) is disputed on the species and subspecies levels (Grubb et al. 2003; Wimberger & Linden 2016) which has led to contradictory and confusing accounts across the literature (Table S.1). Due to availability of samples, genetic studies to date have largely been limited to single specimens per taxon and scattered geographical distribution (Tosi et al. 2005; Guschanski et al. 2013). Dalton et al. (2015) provided larger sample sizes but their study was geographically limited to South Africa. We follow Groves (2001) in recognising *C. albogularis* and *C. mitis* as distinct species.

Cercopithecus albogularis is restricted to different evergreen forest types across its range (Groves 2001; Groves 2005; Kingdon 2013; Lawes et al. 2013). Indigenous forests in southern Africa are naturally highly fragmented from past climatic changes (Eeley et al. 1999), characterised by periodical wet-dry oscillations and a step-like increase of aridity coinciding with an intensification of glacial cycles in the past 2.8 million years (DeMenocal 1995; DeMenocal 2004). These Pleistocene-era climatic oscillations are thought to have played an important role in shaping diversification patterns, particularly at the population level (e.g.

Anthony et al. 2007; Johnston & Anthony 2012). Samango monkey distribution closely follows the patchy forest range which creates a basis for smaller scale genetic population structure (due to isolation and colonisation events). For example, a recent study showed that South African samango monkeys can be divided into three distinct genetic entities: *C. a. labiatus* (Hogsback population; Eastern Cape Province), *C. a. erythrarchus* (Sodwana Bay and Cape Vidal coastal populations; northern KwaZulu-Natal Province) and *C. a. "schwarzi"* (Soutpansberg and northern escarpment populations in Limpopo Province) (Dalton et al. 2015). Considering the clear genetic structure found by that study, we expect this pattern to extend to samango populations elsewhere in southern Africa. The subspecies *erythrarchus* is described to also occur outside of South Africa, from the Mulanje Plateau in southern Malawi, to Zimbabwe, and most of Mozambique (Groves 2001; Kingdon 2013; Lawes et al. 2013). The exact boundary between coastal distributions of *erythrarchus* in northern Mozambique and *C. a. monoides* in southern Tanzania are still unknown (Lernould 1988).

In addition to mainland samango populations in Mozambique, there are several islands along the Mozambique coast, for example Bazaruto (Downs & Wirminghaus 1997), Vamizi (this study) and Matemo (Venter S. pers. comm.), with populations of samangos. Our current study aims to add new mtDNA and microsatellites data from a coastal population (Vamizi Island) in Mozambique to compare to existing data from South Africa (following recommendations by Dalton et al. 2015). Our additional data allow a further test of the number and timing of the radiation events of *Cercopithecus* monkeys in southern Africa as elaborated below. Lawes (1990) suggested two radiation events following the distribution and age of the two main forest types in southern Africa, Afromontane forests and Indian Ocean coastal belt forest (Figure 1a). The former is described as the more ancient (pre LGM) and persistent type (Moreau 1963; White 1981), representing *labiatus* distribution in South Africa (Lawes 1990) and the latter the younger (post LGM) type (Tinley 1985; Mucina et al. 2006), representing *erythrarchus* distribution in South Africa (Lawes 1990). Here we test two alternative hypotheses to explain observed genetic patterns between populations: the multiple radiation hypothesis of Lawes (1990) (Figure 1a) and the single radiation with local *in situ* differentiation hypothesis proposed herein (Figure 1b). Specifically, our new data from Vamizi Island in Mozambique should either group genetically with coastal forest populations in South Africa (hypothesis 1a, node 1; *sensu* Lawes 1990) or with its geographically closest neighbour, Gorongosa (published sequence from Guschanski et al. 2013) (hypothesis 1b, node 4; this study).

Although not the primary focus of this study, we include mtDNA sequence data from *Cercopithecus* across Africa, mostly from Guschansky et al. (2013), to test the hypothesis that the “Kingdon Line” (a faunal boundary line largely dividing the central African lowland and eastern African montane and coastal forests, representing an optimal separation of overlapping central and eastern forest faunal elements) (Grubb et al. 1999; Kingdon 2003; Groves 2015; Joordens et al. 2019) may explain the divergence of two major species in Africa within the *C. mitis/nictitans* group, *C. albogularis* from predominantly coastal and Afromontane forests from south eastern, eastern and north-eastern Africa and *C. mitis* from tropical forested habitats in central and west Africa and N Angola.

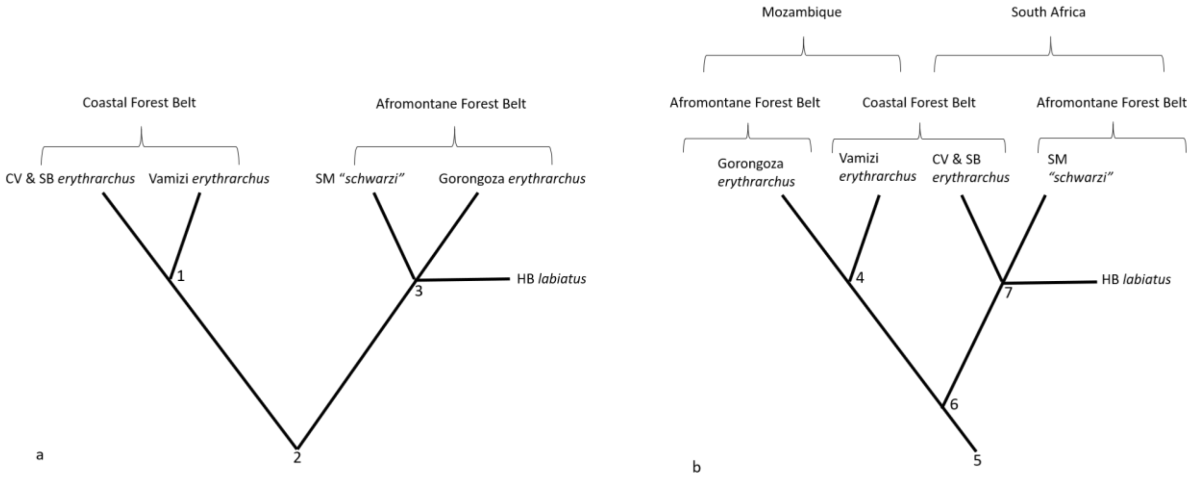

Figure 1. Cladograms illustrating two alternative hypotheses. Cladogram a shows the multiple radiation event hypothesis of Lawes (1990) and cladogram b shows the single radiation with local *in situ* differentiation hypothesis proposed here. Hypothesis a assumes the deepest split between habitat types irrespective of geography (node 2). Hypothesis b assumes the deepest split geographically between countries (node 6). The Gorongosa sample included is of a single *erythrarchus* museum specimen (JQ256960) (Guschanski et al. 2013). Abbreviations follow Dalton et al. (2015): HB = Hogsback, CV = Cape Vidal, SB = Sodwana Bay and SM = Soutpansberg Mountains.

2. Methods

2.1. Sample collection

In Mozambique, we collected samples of five samango monkeys opportunistically from deceased individuals at the Vamizi Island Lodge, Palma District, Cabo Delgado Province. The suspected cause of death was rat poison, since the new rat control team initially did not use available monkey proof bait boxes. Tissue and skin samples were preserved in 90% ethanol.

We obtained relevant permission for collecting, importing and storing the samples (CITES Permit No. 142706; Department of Environmental Affairs (South Africa) Standing Permit No. S07507). Sequences are available on the GenBank database under accession numbers: MK933732, MK933734, MK920178-MK920181 and MK940914-MK940917.

2.2. Microsatellite genotyping and analysis

In this study, we genotype five samples from Mozambique (MZ; -11.11246, 40.63984) at 21 microsatellite loci following methods described in Dalton et al. (2015). Microsatellite and mitochondrial data were additionally available from four populations in South Africa (Dalton et al. 2015): *C. a. labiatus* from (1) Hogsback (HB; -32.59526, 26.95675, n = 37) and *C. a. erythrarchus* from (2) Cape Vidal (CV; -28.12329, 32.55638; n = 5), (3) Sodwana Bay (SB; -27.54692, 32.66939; n = 11) and (4) Soutpansberg mountains (SM; -23.03788, 29.44195, n = 16) (Figure 2). Thus, the total dataset included 74 individuals from five populations.

We used MICRO-CHECKER (Van Oosterhout et al. 2004) to detect potential genotyping errors, allele dropout and non-amplified alleles. We used MS TOOLKIT (Park 2001) and GENALEX v.6.5 (Peakall and Smouse 2006; Peakall and Smouse 2012) to estimate the levels of genetic diversity within populations, mean number of alleles per locus (N_A), observed heterozygosities (H_O), expected heterozygosities (H_E and uH_E) and deviations from Hardy-Weinberg equilibrium (HWE). We evaluated linkage disequilibrium between pairs of microsatellite loci within each population and locus using GENEPOP v.4.1.4 (Raymond & Rousset 1995; Rousset 2008) and associated probability values were corrected for multiple comparisons using Bonferroni adjustment for a significance level of 0.05. F_{ST} analyses of molecular variance (AMOVA) (Excoffier et al. 1992) were carried out in GENALEX to estimate levels of divergence among populations.

We used STRUCTURE v.2.3.3 (Pritchard et al. 2000) to infer the genetic relationship between populations via a Bayesian clustering analysis where runs were done without prior population information (option USEPOPINFO=0, no LOCPRIOR) to ensure that pre-defined populations were in agreement with the genetic data. We initially ran STRUCTURE for all samples with 20 replicates, K = 1-9, 1 million repetitions of Markov Chain Monte Carlo (MCMC) and a burn-in of 100 000 iterations. The K with the greatest increase in posterior probability (ΔK) (Evanno et al. 2005) was identified as the optimum number of sub-populations using STRUCTURE HARVESTER (Earl & vonHoldt 2012). CLUMPP (Jakobsson & Rosenberg 2007) generated the average of these runs for the appropriate K, and results were visualised in

DISTRUCT (Rosenberg 2004). A second STRUCTURE run that included only 35 C. a. erythrarchus individuals was run and the settings were 20 replicates, K = 1-5, 1 million MCMC, 100 000 burn-in, admixture model and no LOCPRIOR. STRUCTURE HARVESTER identified the most likely number of clusters. We averaged results using CLUMPP and visualised the results using DISTRUCT.

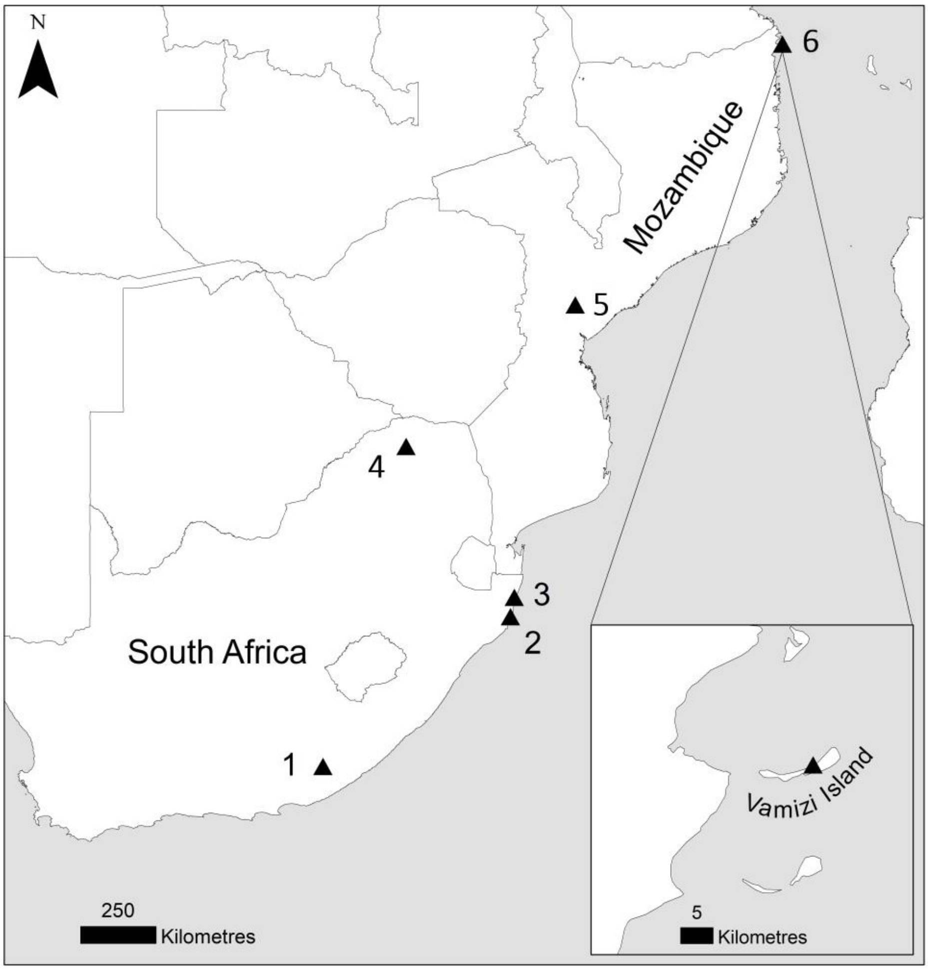

Figure 2. Sampling localities of samango populations from southern Africa including Dalton et al. (2015) samples from South Africa: Hogsback (1), Cape Vidal (2), Sodwana Bay (3), Soutpansberg Mountains (4); the Guschansky et al. (2013) museum sample (JQ256960) from Gorongosa (5) and samples acquired for this study from Vamizi Island (6).

2.3. mtDNA sequencing, analysis and divergence dating

Here, two mitochondrial gene regions (Cyt B and 16S) were sequenced for four MZ samples using methods presented in Dalton et al. (2015). In addition, D-loop sequences were generated for 21 samples (7 x HB, 2 x CV, 5 x SB, 4 x SM from Dalton et al. (2015) and 3 x MZ from this study) using primers and protocols from Meyer et al. (2011). Resulting sequence chromatograms were viewed and edited in the Chromas program embedded in MEGA6 (Tamura et al. 2013). We placed Cyt B and 16S sequences in a concatenated alignment of 910 nucleotides, whereas D-loop consisted of an alignment of 408 nucleotides. Additional *C. a. labiatus* (JQ256961) (Eastern Cape, South Africa) and *C. a. erythrarchus* (JQ256960) (Gorongosa, mainland Mozambique) samples were included with the ingroups. Phylogenetic relationships were evaluated using the maximum likelihood (ML) method and Hasegawa-Kishino-Yano model implemented in MEGA6 (Hasegawa et al., 1985; Tamura et al. 2013). To estimate support for internal nodes, 1 000 bootstrap replications were run using the same program (Felsenstein 1985; Tamura et al. 2013). *C. erythrotis erythrotis* (JQ256935) was used as an outgroup.

We performed Bayesian implementation of rate variation in BEAST (Drummond & Rambaut 2007) using concatenated data to provide a representation of the evolutionary phylogenetic relationships and establish the divergence time of the various South African and Mozambican *Cercopithecus* groupings. We used the GTR+G substitution model, selected under the Akaike Information Criterion (AIC) in jModeltest 0.1.1 (Guindon & Gascuel 2003; Posada 2008), with estimated base frequencies. We estimated mean and 95% confidence limits of specified nodal dates within Cercopithecidae via Markov Chain Monte Carlo [MCMC] algorithm, with a chain length of 5 million, sampled every 1 000 iterations with burn-in of 500 000. We performed analysis under the uncorrelated relaxed clock model (branch-specific rates followed a log-normal distribution) using BEAST v. 1.6.1 (Drummond & Rambaut 2007) in conjunction with BEAUti v.1.6.1 and Tracer v. 1.5 (Rambaut & Drummond 2009). In order to accurately conduct molecular dating, the use of reliable fossil calibration points is critical (Lee 1999). Here, we followed the method of Guschanski et al. (2013) for phylogenetic reconstruction and dating and used calibration points based on undisputed fossils as the outgroup taxa (*Microcolobus tugenensis* (Benefit and Pickford 1986) (10 MYA), *Macaca libyca* (6 MYA), *Theropithecus gelada* / *Papio hamadryas* (4.32 MYA) and *Cercocebus chrysogaster* / *C. atys* (3.39 MYA). We calibrated nodes using normal distribution priors and set standard deviation at 0.5. We used Tree Annotator v. 1.6.1 (Drummond & Rambaut 2007) to find the best supported tree which we then viewed and edited in FigTree v.1.3.1 (Rambaut 2009).

3. Results

3.1. Microsatellite analysis

Deviations from HWE equilibrium were not consistent across populations, with significant deviations from HWE only being observed in two markers (D12S67 and D2S1326) in the HB and SB populations and in one marker (D4S243) in the SM population. Departures from HWE in these populations may be due to unrecognised populations subdivision or inbreeding. GENEPOP identified six pairs of loci as significantly linked over all populations which may be due to substructure, small population sizes and/or bottlenecks (Hartl & Clark 1997). Analysis with and without linked loci were similar suggesting that the presence of LD did not influence these results. The mean number of alleles (N_A) and mean expected heterozygosity (H_E) across all populations and loci was 3.0 and 0.44, respectively (Table 1) with the highest H_E and N_A found in the SB population. Variability in the Mozambique samples ($H_E = 0.43$) was similar to that of Cape Vidal animals ($H_E = 0.42$) and lower than the Sodwana Bay individuals ($H_E = 0.52$). We observed private alleles in all populations: 17 in HB, 5 in SB and CV combined, 3 in SM and 12 in MZ.

Table 1. Genetic variation estimates. The number of alleles (N_A±SE), number of effective alleles (N_{eA}), observed (H_O) and expected (H_E) heterozygosities, and unbiased expected heterozygosity (uH_E) over all loci. Populations are defined as the currently recognised subspecies (in bold) as well as among five populations of samango monkeys. * Indicates samples analysed in this study.

Subspecies	**N_A**	**N_{eA}**	**H_O**	**H_E**	**uH_E**
C. a. labiatus	**3.79±0.42**	**1.89±0.17**	**0.40±0.06**	**0.39±0.06**	**0.39±0.06**
Hogsback (*C. a. labiatus*)	3.79±0.42	1.89±0.17	0.40±0.06	0.39±0.06	0.39±0.06
C. a. erythrarchus	**5.53±0.43**	**3.30±0.33**	**0.45±0.04**	**0.64±0.04**	**0.65±0.04**
Soutpansberg (*C. a. "schwarzi"*)	2.84±0.33	1.98±0.18	0.42±0.06	0.42±0.05	0.44±0.05
Mozambique (*C. a. erythrarchus*)*	2.53±0.28	2.12±0.22	0.30±0.06	0.43±0.06	0.49±0.06
Cape Vidal (*C. a. erythrarchus*)	2.42±0.22	1.94±0.15	0.48±0.08	0.42±0.05	0.47±0.06
Sodwana Bay (*C. a. erythrarchus*)	3.53±0.31	2.50±0.25	0.55±0.07	0.52±0.05	0.55±0.05
Total (mean±SE)	**3.02±0.15**	**2.09±0.09**	**0.43±0.03**	**0.44±0.02**	**0.47±0.03**

To investigate population structuring, we interpreted data using the ΔK method (Evanno et al. 2005), where $K = 2$ yielded the highest ΔK, grouping populations into *C. a. erythrarchus* and *C. a. labiatus* (Figure 3a). Further structure analysis of *C. a. erythrarchus* individuals identified the most likely number of clusters as $K = 3$, reflecting their collection localities in (1) the Soutpansberg Mountains, (2) Sodwana Bay and Cape Vidal and (3) Mozambique (Figure 3b

& c). A total of 33% of variance is explained among the five populations and 6% among individuals. Pairwise F_{ST} values estimated using AMOVA (Table 2) show that the HB population is consistently significantly and highly differentiated from all other populations. F_{ST} values showed the closest relationship to be between CV and SB populations (0.190) and the most distant between CV and HB populations (0.410). Within *C. a. erythrarchus,* the CV population is most genetically different from the Mozambique population.

Table 2. Microsatellite-based pairwise FST values estimated during AMOVA (below diagonal) and D-loop-based pairwise p-distances (above diagonal).

Population	**HB**	**SB**	**CV**	**SM**	**MZ**
Hogsback (HB; *C. a. labiatus*)	-	0.081	0.100	0.106	0.089
Sodwana Bay (SB; *C. a. erythrarchus*)	0.275	-	0.051	0.051	0.073
Cape Vidal (CV; *C. a. erythrarchus*)	0.410	0.190	-	0.083	0.109
Soutpansberg Mountains (SM; *C. a.”schwarzi”*)	0.361	0.281	0.363	-	0.086
Mozambique (MZ; *C. a. erythrarchus*)	0.370	0.252	0.390	0.373	-

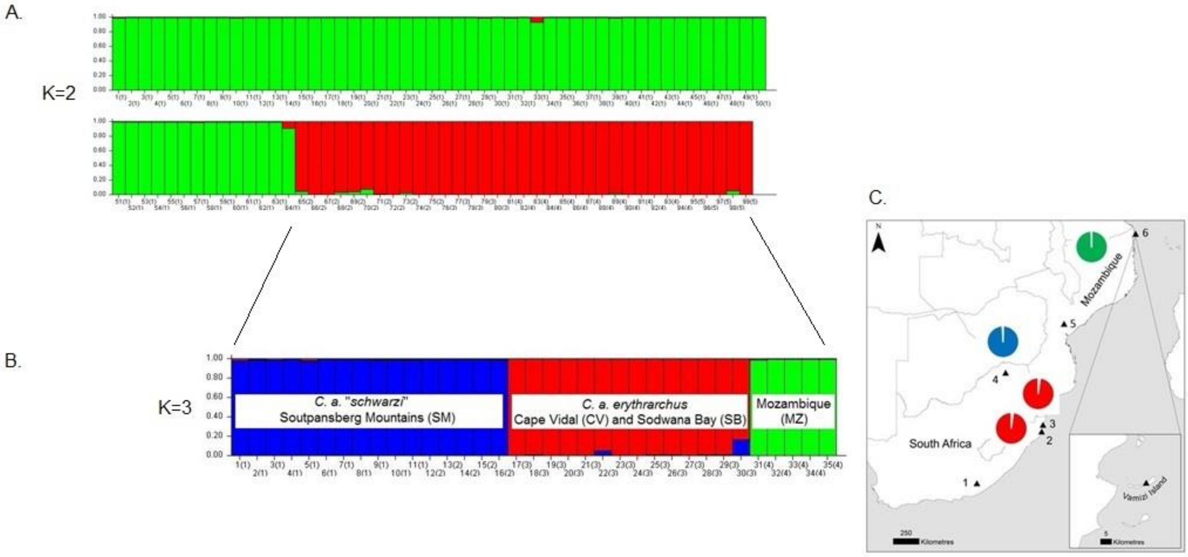

Figure 3. A. Bayesian assignment probabilities for K = 2 of microsatellite genotypes of *C. a. erythrarchus* and *C. a. labiatus.* B. Bayesian assignment probabilities for K = 3 of microsatellite genotypes of *C. a. “schwarzi”* (Soutpansberg Mountains, SM), *C. a. erythrarchus* (Cape Vidal, CV and Sodwana Bay SB) and *C. a. erythrarchus* (Vamizi Island, Mozambique, MZ). C. Distribution of cluster assignment percentages (in pie-charts) among populations for K = 3. Coloured pie charts show the average cluster membership of individuals.

3.2. mtDNA analysis

The phylogenetic trees we obtained from the ML analyses for D-loop (Figure 4) and Cytb/16S (Figure 5) shows the monophyletic separation of *C. albogularis* spp. from the *C. e. erythrotis* outgroups. Branching patterns place the CV and SB populations as sister to each other (67% bootstrap for D-loop and 46% bootstrap for Cytb/16S), with SM sister to this CV/SB grouping, albeit with lower support (30 to 58% bootstrap). Separation of the *C. a. labiatus* HB population from the CV/SB/SM grouping is supported with 83% bootstrap for Cytb/16S and 23% for D-loop and MZ individuals appear to be relatively distinct from the CV/SB/SM/HB cluster. D-loop pairwise p-distances support these findings where the highest net distance (10.9%) separates MZ *C. a. erythrarchus* and CV *C. a. erythrarchus* (Table 2). Distances between the remaining *C. a. erythrarchus* (SB, CV), *C. a. "schwarzi"* (SM) and *C. a. labiatus* (HB) populations ranged from 8.1% – 10.6%.

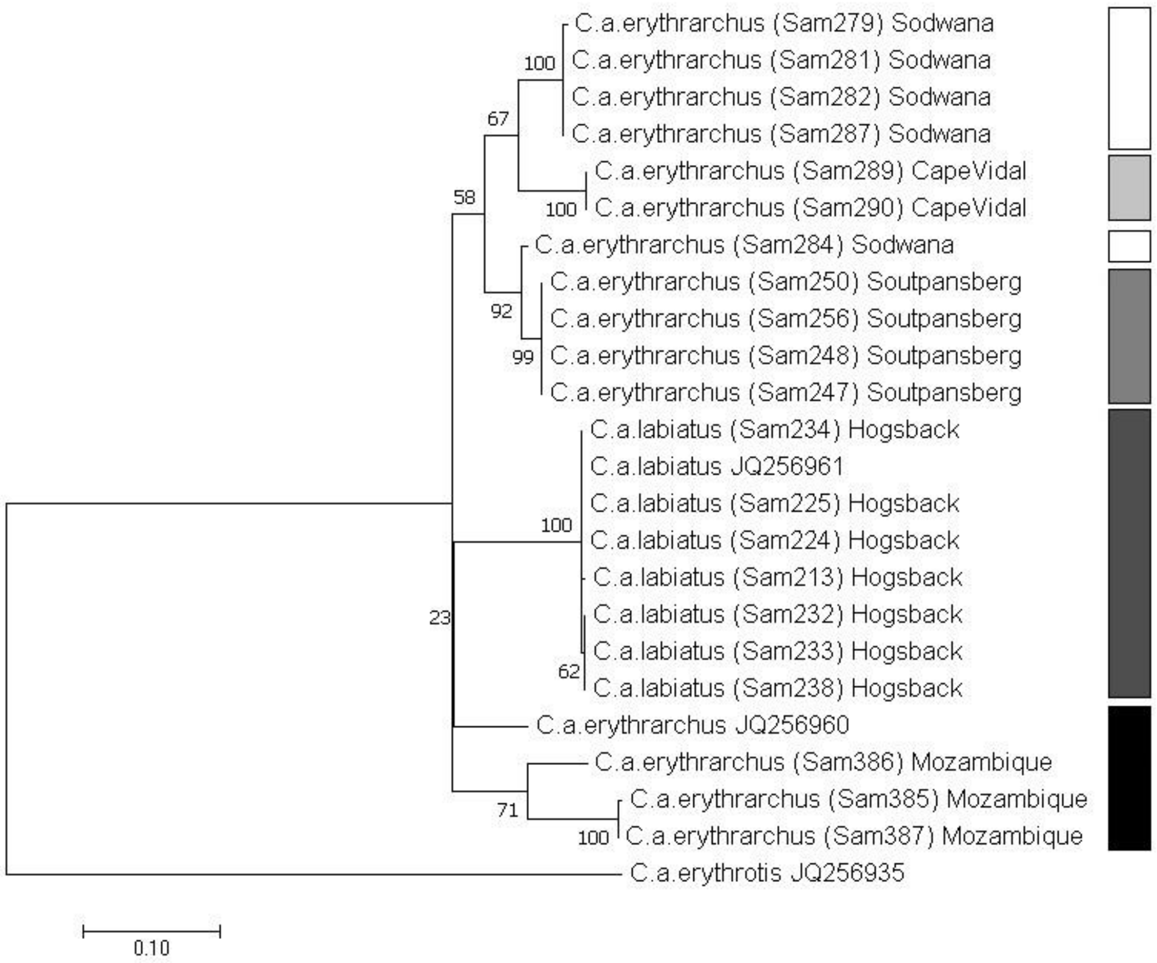

Figure 4. Molecular phylogenetic analysis by a maximum likelihood method using D-loop gene sequences. The evolutionary history was inferred by using the maximum likelihood

method based on the Hasegawa-Kishino-Yano model with 1 000 bootstrap replications. The tree with the highest log likelihood (-2443.05) is shown. The percentage of trees in which the associated taxa clustered together is shown next to the branches. Initial tree(s) for the heuristic search were obtained automatically by applying Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using the Maximum Composite Likelihood (MCL) approach, and then selecting the topology with superior log likelihood value. A discrete Gamma distribution was used to model evolutionary rate differences among sites (5 categories (+G, parameter = 0.5416)). The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. This analysis involved 24 nucleotide sequences. There was a total of 558 positions in the final dataset. Evolutionary analyses were conducted in MEGA6.

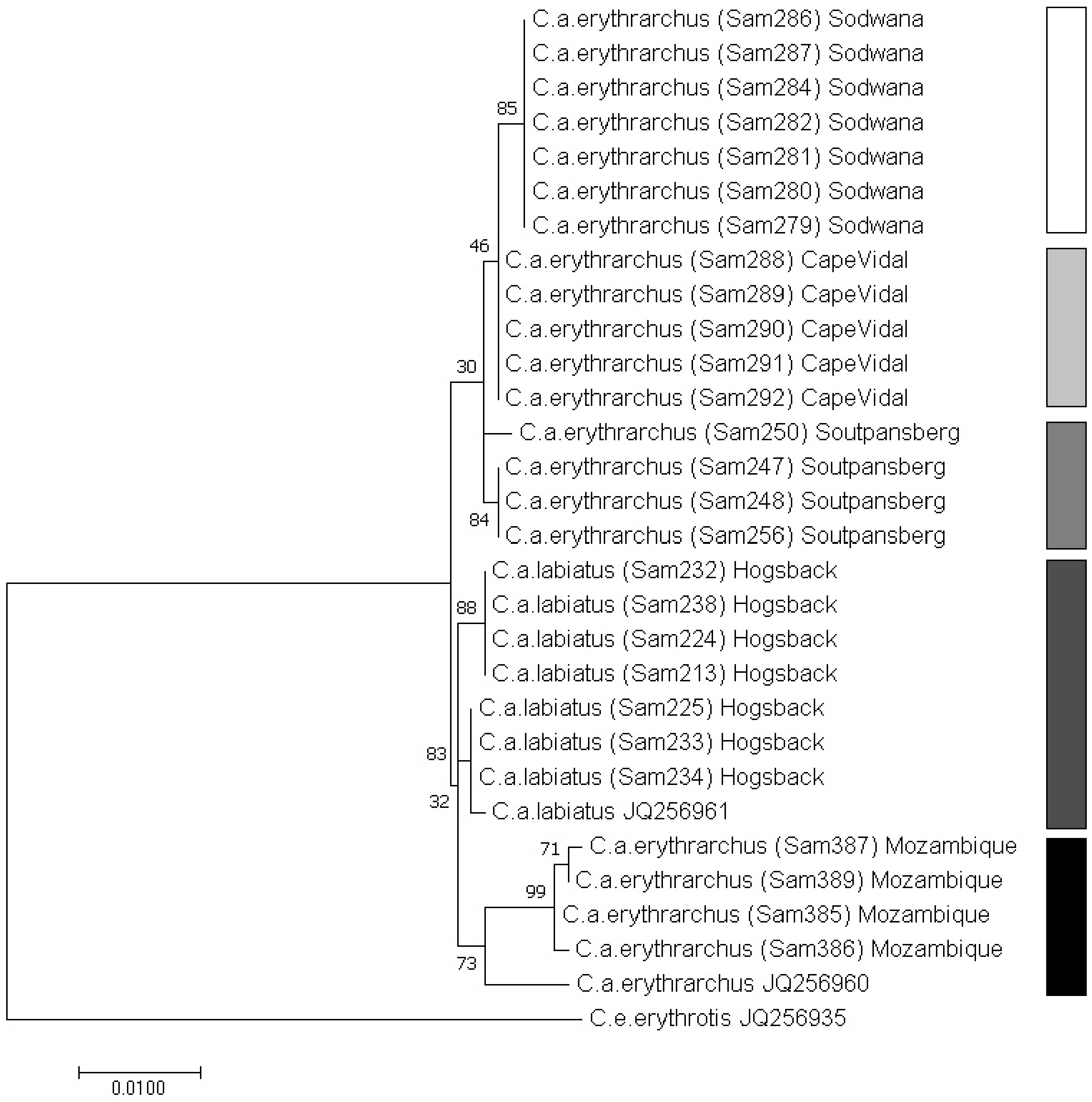

Figure 5. Molecular phylogenetic analysis by a maximum likelihood method using concatenated Cyt B and 16S gene sequences. The evolutionary history was inferred by using the Maximum Likelihood method and Hasegawa-Kishino-Yano model. The tree with the highest log likelihood (-1936.10) is shown. The percentage of trees in which the associated taxa clustered together is shown next to the branches. Initial tree(s) for the heuristic search were obtained automatically by applying Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using the Maximum Composite Likelihood (MCL) approach, and then selecting the topology with superior log likelihood value. A discrete Gamma distribution was used to model evolutionary rate differences among sites (5 categories (+G, parameter = 0.0679)). The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. This analysis involved 30 nucleotide sequences. There was a total of 913 positions in the final dataset. Evolutionary analyses were conducted in MEGA6.

Divergence dates for major nodes, including within *Cercopithecus*, are given in Table 3 and are represented in Figure 6. The *C. e. erythrotis* clade was separated from the remaining *C. albogularis* and *C. mitis* taxa at 4.89 MYA (node I; 95% confidence limits: 3.73 – 6.28 MYA). Node G dates the separation of *C. albogularis* sensu Groves (2001), comprising all Afromontane and coastal forest forms from south-central, eastern and north-eastern Africa, from *C. mitis* sensu Groves (2001), in part (*doggetti, stuhlmanni, kandti, kolbi, haymanae, francescae*), comprising tropical forest forms from central Africa. This division coincides geographically and ecologically (between coastal/Afromontane and tropical forests) very closely with the "Kingdon Line" (Figure S.1). Node H dates the separation between a western group of tropical forest *C. mitis* (*mitis*, *nictitans*, *opisthosticus*) and a group combining *albogularis* and the eastern group of *mitis* mentioned above. Node F dates the separation of South African/Mozambique *C. albogularis* from a south-central, eastern and north-eastern Africa montane and coastal forest clade comprising *C. a. moloneyi*, *C. a. albogularis, C. a. monoides*, *C. a. albotorquatus* and *C. m. boutourlinii* at 1.02 MYA (95% confidence limits: 0.73 – 1.40 MYA). The split between Mozambican and South African *C. albogularis* clades is dated at 0.71 MYA (node D; 95% confidence limits: 0.44 – 1.08 MYA). Within the South African clade, *C. a. labiatus* (HB) split from *C. a. erythrarchus* (SB, CV) / *C. a. "schwarzi"* (SM) 0.55 MYA (node C; 95% confidence limits: 0.34 – 0.82 MYA); the Sodwana *C. a. erythrarchus* and Soutpansberg *C. a. "schwarzi"* / Cape Vidal *C. a. erythrarchus* groups split 0.43 MYA (node B; 95% confidence limits: 0.23 – 0.68 MYA) and Soutpansberg *C. a. "schwarzi"* and Cape Vidal *C. a. erythrarchus* split 0.3 MYA (node A; 95% confidence limits: 0.14 – 0.51 MYA) (Table 3, Figure 6).

Table 3. Divergence date estimates (MYA) inferred using BEAST v.1.6.1 for nodes A – I from Figure 4. Divergence dates were obtained from Stromer (1920), Benefit & Pickford (1986) and Guschanski et al. (2013) for analysis. The taxonomy herein follows Groves (2001) who recognised C. mitis and C. albogularis as distinct species (and not sections or clusters as in Kingdon 2013, Lawes et al. 2013).

Taxon-set	**Mean node date**	**95% confidence interval**	**Node**	**Comment**
Cape Vidal *C. albogularis erythrarchus*	0.30	0.14 – 0.51	A	Node dividing *C. a. erythrarchus* from Cape Vidal and *C. a. "schwarzi"* from Soutpansberg
Sodwana *C. a. erythrarchus*	0.43	0.23 – 0.68	B	Node separating Sodwana *C. a. erythrarchus* from Cape Vidal *C. a. erythrarchus* / Soutpansberg *C. a. "schwarzi"*
Hogsback *C. a. labiatus*	0.55	0.34 – 0.82	C	Node separating Hogsback *C. a. labiatus* from South African *C. a. erythrarchus* (Cape Vidal/ Sodwana Bay) and *C. a. "schwarzi"* (Soutpansberg)
*C. a. erythrarchus*_JQ256960	0.71	0.44 – 1.08	D	Node separating Mozambican *C. a. erythrarchus*_JQ25690 (Guschanski et al., 2013) from South African *C. a. erythrarchus / C. a. "schwarzi" / C. a. labiatus* samples
Mozambique *C. a. erythrarchus*	0.86	–	E	Node separating Mozambican *C. a. erythrarchus* (this study) from Mozambican *C. a. erythrarchus*_JQ25690 (Guschanski et al., 2013) and South African *C. a. erythrarchus / C. a. "schwarzi" / C. a. labiatus* samples
C. albogularis sensu Groves 2001	1.02	0.73 – 1.40	F	Node separating Zambian/Tanzanian (*monoides*, *moloneyi*, *albogularis*), E Kenyan (*albotorquatus*) and Ethiopian (*boutourlinii*) forms of *C. albogularis* from all South African / Mozambican *C. albogularis*
C. mitis sensu Groves 2001(east group)	2.57	1.94 – 3.28	G	Node separating *C. albogularis* from central African forms of *C. mitis* according to the "Kingdon Line" (see text)
C. mitis sensu Groves 2001 + *nictitans* (west group)	3.26	2.50 – 4.13	H	Node separating *C. mitis* western subgroup from *C. mitis* eastern subgroup
C. erythrotis	4.89	3.73 – 6.28	I	Node dividing *C. erythrotis* from remaining *Cercopithecus* species

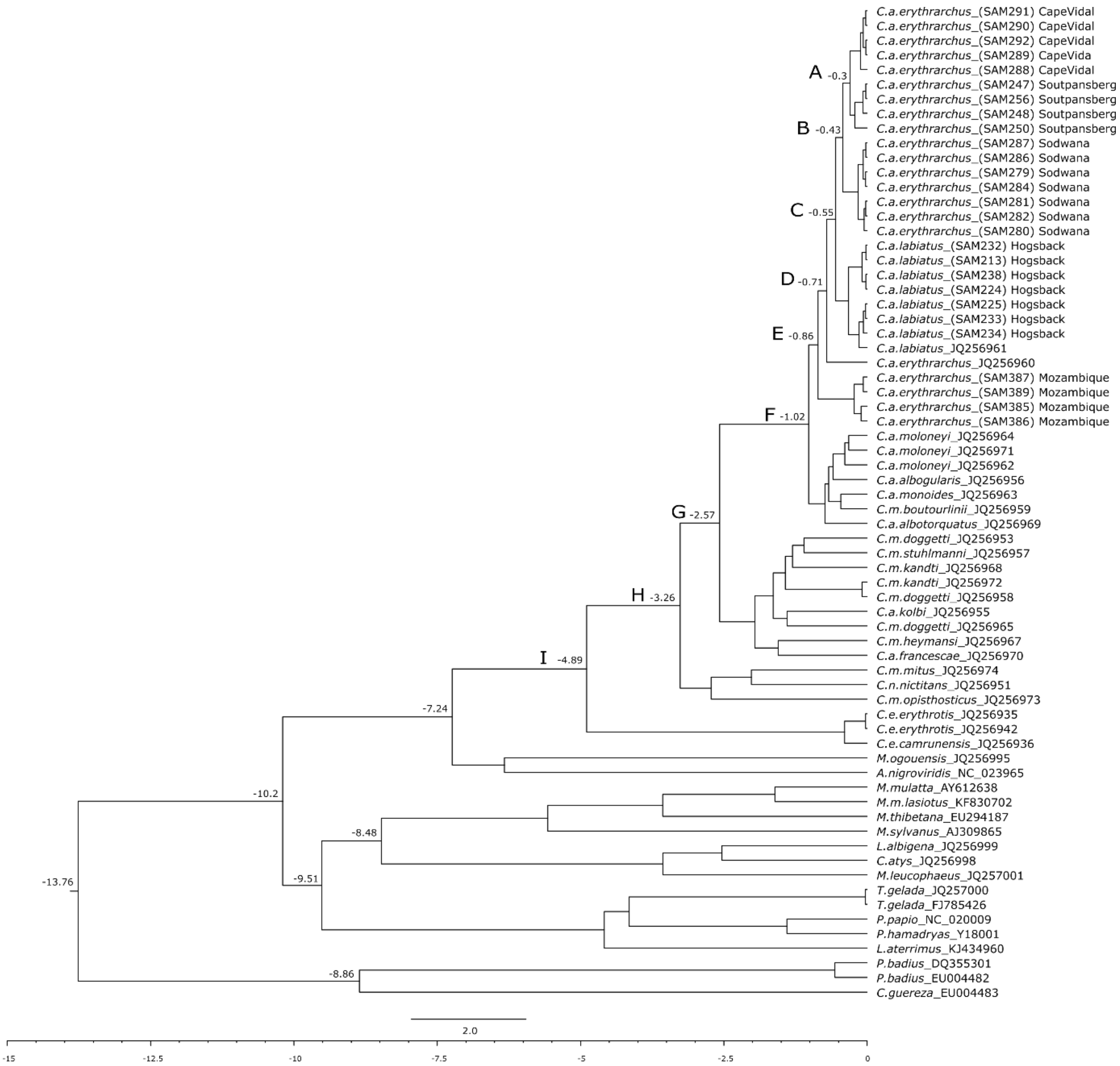

Figure 6. Divergence dated tree based concatenated data. Tree depicts relationships between Cercopithecoidea haplotypes with reference to non-Cercopithecoidea outgroups with dates of nodes given in millions of years ago (MYA).

4. Discussion

4.1. Genetic structuring of populations

Genetic diversity between recognised subspecies (*C. a. labiatus, C. a. "schwarzi"* and *C. a. erythrarchus*) varied in terms of the number of alleles (2.83 to 3.79) and heterozygosity (H_E: 0.39 to 0.46). Lower genetic diversity was observed in the HB population (H_E = 0.39) with the highest diversity being observed in the SB group (H_E = 0.52). In the present study, we support previous findings of Dalton et al. (2015) and Madisha et al. (2017), indicating that samango monkeys harbour less genetic diversity (H_E: 0.44) compared to other species in the genus;

namely Campbell's monkey (*C. campbelli*) with a reported H_E of 0.74 (Petit et al. 2010) and compared to other primates affected by habitat fragmentation for example H_E values (0.66–0.75) reported for orang-utan (*Pongo pygmaeus*, Goossens et al. 2005) and an H_E value of 0.72 described for red colobus (*Procolobus tephrosceles*; Allen et al. 2012). Lower variability is associated with smaller populations and can additionally be related to an evolutionary history of population isolation due to forest fragmentation (Pan & Jablonski 1987). Although moderate genetic diversity was observed in Mozambique individuals from Vamizi Island, this population may be at risk of on-going genetic diversity loss due to anthropogenic pressures such as habitat transformation and geographic isolation (Isabel Silva, pers. comm.). As genetic diversity is required for species to adapt to environmental change (Frankham 1995; Frankham et al. 2002), potential consequences of further genetic diversity loss in this population is of conservation concern. The results obtained from AMOVA between MZ and HB/SM/CV/SB, where an average F_{ST} of 0.346 (Table 2) is given, supports genetic differentiation between the Mozambican samples (MZ) in relation to other South African *C. albogularis* populations sampled here. This separation is well supported by STRUCTURE analysis which shows the separation of *C. a. erythrarchus* into two distinct groupings, namely a MZ group and CV and SB group (Figure 3). In addition, we detected several private alleles. The observed differentiation of the MZ group may be attributed to island population effects whereby island populations have lower gene flow due to reduced migration resulting in genetic structure and increased levels of inbreeding and increased chance of genetic drift (Frankham 1997, 1998). However, although mainland Mozambique samples were not available for the microsatellite analysis, mtDNA sequences were accessible from a single mainland individual. Here, the mainland and island MZ samples in the mtDNA maximum likelihood trees are placed in a distinct cluster (Figures 4 & 5), with moderate node support (D-loop tree 71% and Cytb/16S tree 73%). Thus, it is likely that gene flow via migration is potentially still occurring or has occurred in the recent past between mainland and island MZ individuals. This is supported by the observed pairwise genetic p-distances of 8.9% as well the absence of loss of genetic diversity in MZ ($H_E = 0.43$) in comparison to the SM ($H_E = 0.42$), CV ($H_E = 0.42$) and SB ($H_E = 0.52$) populations. The moderate node support can further be indicative for a rapid north-south radiation with subsequent differentiation. Despite moderate node support, dating analysis places the divergence of the MZ population from South African populations at 0.71 MYA – 0.86 MYA (depending on whether or not one includes the single Gorongosa sample), which is prior to the separation of the *C. a. labiatus* (HB) population from the South African *C. a. erythrarchus* (SB, SM and CV) populations (0.55 MYA). The divergence of *C. a. labiatus* and

C. a. erythrarchus reported here is somewhat later than previously proposed at 1.7 MYA (Dalton et al. 2015). However, further investigation that includes additional individuals and/or genetic markers should be employed to ascertain the evolutionary relationships among these closely related subspecies.

4.2. Phylogeography

According to Lawes (1990) one should expect *erythrarchus* populations from the coastal belt forests in South Africa to genetically cluster with populations from the coastal belt forests in Mozambique if they form part of the same (post LGM coastal belt forest) radiation event. However, results from our study show that *erythrarchus* from Vamizi Island (coastal belt) and Gorongosa (Afromontane) in Mozambique are basal to all the South African samples, diverging about 0.71 – 0.86 MYA, long before the LGM. The hypothesis of Lawes (1990) of two separate radiations events into South Africa, one before (*labiatus*) and one after (*erythrarchus*) the LGM, is not supported by our data. Rather, our data support the hypothesis of a single mid-Pleistocene unidirectional, north-south, radiation event into South Africa. Cercopithecines are a highly polymorphic and diverse group and the hypothesis that Cercopithecini species groups radiated during the Pleistocene was first formulated by Booth (1958) and later supported through molecular studies by Tosi et al. (2005) and Tosi (2008). The situation is reminiscent of the pattern found in Afro-temperate forest shrews *(Myosorex)* where the eastern Zimbabwe population (*M. meesteri)* was basal to all South African populations of *M. varius* and *M. cafer*, also suggesting a sequential north-south pattern of migration during early to mid-Pleistocene followed by subsequent isolation (Taylor et al. 2013). Further, Johnston and Anthony (2012), in their phylogenetic study on African forest duikers (Cephalophinea), showed a fairly recent radiation of a group of duikers whose sister species pairs appear to date predominantly to the Pleistocene, highlighting the potential importance Pleistocene refugia may have played in the speciation of forest-dwelling species (overview of the Pleistocene Refugia Hypothesis e.g. in Mayr & O’Hara, 1986). Our data also refute Kingdon’s (2013) notion of a very widespread relictual distribution of the *C. mitis* group with conservative, morphologically similar ancestral morphs existing at southern (*labiatus*, South Africa) and northern (*zammaranoi*, Somalia and *boutourlinii*, Ethiopia) extremes. Our data show that *labiatus* diverged from *erythrarchus* and “*schwarzi*” at around 0.55 MYA subsequent to the split between Mozambique and South African populations (0.71 – 0.86 MYA). If *labiatus* was an older relictual population it would have been genetically distinct from its geographically closest neighbours and maintained genetic links with geographically distant populations representing the original radiation event.

Southern Africa climate during the LGM was markedly drier and colder than today. It is suggested that rainfall was 40–70% below today's annual average and that temperatures were 5-6 ºC lower (Deacon 1983; Mucina et al. 2006). Assuming that the coastal belt forest (re)established after the LGM (Tinley 1985; Mucina et al. 2006) and using our data showing that South African *erythrarchus* forms a separate clade from Mozambican *erythrarchus,* we further conclude that the radiation event into South Africa in the mid-Pleistocene must have mainly occurred along the Afromontane forest belt in which populations persisted throughout the LGM and from which they subsequently re-radiated into more recently (re)established coastal belt forests during the past 10 000 years or so. Coastal scarp forest patches in South Africa may have also persisted, offering refugia for forest fauna (Lawes 1990). During the past 0.8 million years, the climate was characterised by glacial-interglacial oscillations comprising 11 interglacial periods (Past Interglacials Working Group of PAGES 2016). The radiation of samango monkeys from Mozambique into South Africa around 0.71 MYA – 0.86 MYA occurred during one of the interglacial periods, MIS 17 (Marine Isotope Stage 17). Pleistocene climatic conditions in subtropical southern Africa are still comparatively understudied although recent research by Castañeda et al. (2016) suggests that Pleistocene environments in southern Africa were generally more stable compared to those of tropical Africa, providing more consistently suitable environments for fauna throughout the Pleistocene at times when tropical eastern Africa experienced extreme aridity. They further suggest that interglacial periods in southern East Africa were characterised by relatively wetter conditions, with a slight increase of C3 (trees, shrubs, some grasses) vegetation. Dupont et al. (2011) analysed terrestrial pollen assemblages from the Limpopo River mouth spanning the past 325 ka years and suggested that the vegetation of eastern South Africa and southern Mozambique largely consisted of evergreen and deciduous forests during interglacial periods with montane *Podocarpus* forest extended during humid periods. Glacial periods are generally suggested to have been more arid with an increase of grasses (C4 vegetation) as well as open mountain vegetation dominated by ericaceous scrubs (Ericaceae and some Asteroideae) (Dupont et al. 2011; Castañeda et al. 2016). This leads us to the assumption that similar vegetation patterns of increased forest cover occurred at the time of divergence of South African and Mozambican samango monkeys enabling them to radiate as far south as the Eastern Cape.

Literature on the formation and age of Vamizi Island is scant but studies including paleo digital elevation models on global sea level fluctuations indicate that at the peak of the LGM (21 000

B.P.) the sea level had receded to the extent that Vamizi was part of the mainland (Figure S.1; Karger et al. 2017) and that sea levels returned to near present coastline about 7 000 B.P. (Tinley 1985; Lambeck et al. 2002). This means that Vamizi Island and its samango monkey population was isolated from the mainland around 7 000 years ago and thus more recent gene flow maintaining genetic diversity was only possible through sea passage after that time. If samango monkeys can cross the 3.7+ km gap between the mainland and the island by themselves, or if human settlers brought individuals onto the island during the past at least 500 years since first settlement (Newitt 1995) can only be answered once further genetic samples from Mozambique mainland populations (just south of the Ruvuma River in Niassa and Cabo Delgado Provinces and south of the Zambezi River, geographically closer to *erythrarchus* type locality in Inhambane) become available. These together with samples from north of the Ruvuma River (southern Tanzania) will also aid resolving the current uncertainty of the subspecies boundaries between *erythrarchus* and *monoides*.

At a continental scale, as also supported by Guschansky et al. (2013), our data support Groves's (2001) recognition of *C. albogularis* and *C. mitis* as two distinct species. As geographically delineated by Node G in Figure 5, we propose that these two species split around 2.5 MYA along the "Kingdon Line" (Figure S.2), with more patchily distributed coastal forest and Afromontane forest populations occurring to the east and more continuously distributed tropical forest populations occurring to the west.

5. Conclusion

Concerning the two alternative hypotheses proposed for the radiation of southern African samango monkeys (Figure 1), our data seem to strongly support a single north-south Pleistocene radiation event (Figure 1b) rather than multiple radiation events occurring before and after the LGM in the recent Holocene (Figure 1a). South African and Mozambican populations diverged about 0.71 MYA during a colder interglacial period and subsequent events have differentiated South African populations of *C. a. labiatus*, *C. a. "schwarzi"* and *C. a. erythrarchus* from isolated forests in the Eastern Cape, northern Limpopo and eastern KwaZulu-Natal provinces respectively. The three taxa are highly structured genetically, and the highly fragmented nature of the species forest habitat has ensured that populations in isolated refuges have diverged genetically over long periods of evolution in spite of climatic oscillations associated with glacial and interglacial periods in the Pleistocene. Nevertheless, just a few of these glaciation events led to expansion of suitable forest habitats and radiation of the forms above. Probably a similar process has occurred throughout the southern and eastern African range of *C. albogularis* giving rise to a variety of geographically delineated and divergent lineages. Further sampling is required to define the boundaries of these lineages, and their taxonomic resolution remains unclear.

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Supplementary

Table S.1. Classifications of the *Cercopithecus mitis* subgroup by various authors since 2001 with names of species, sections and clusters in capitals. For classifications of this subgroup prior to 2001 see Grubb et al. (2003).

Groves, 2001	**Grubb et al., 2003**	**Lawes et al. 2013**	**Kingdon, 2013**
***Cercopithecus* species**	**Sections of *C. mitis***	**Section of *C. mitis***	**Cluster of *C. nictitans***
ALBOGULARIS	ALBOGULARIS	ALBOGULARIS	ALBOGULARIS
C. a. albotorquatus	*C. m. albotorquatus*	*C. m. albotorquatus*	*C. (n.) a. albotorquatus*
C. a. zammaranoi	synonym of *albotorquatus*	*C. m. zammaranoi*	*C. (n.) a. zammaranoi*
C. a. phylax	synonym of *albotorquatus*	synonym of *albotorquatus*	synonym of *albotorquatus*
C. a. kolbi	*C. m. kolbi*	*C. m. kolbi*	*C. (n.) a. kolbi*
C. a. albogularis	*C. m. albogularis*	*C. m. albogularis*	*C. (n.) a. albogularis*
C. a. kibonotensis	synonym of *albogularis*	synonym of *albogularis*	synonym of *albogularis*
C. a. monoides	*C. m. monoides*	*C. m. monoides*	*C. (n.) a. monoides*
C. a. erythrarchus	*C. m. erythrarchus*	*C. m. erythrarchus*	*C. (n.) a. erythrarchus*
C. a. schwarzi	synonym of *erythrarchus*	synonym of *erythrarchus*	synonym of *erythrarchus*
C. a. labiatus	*C. m. labiatus*	*C. m. labiatus*	*C. (n.) a. labiatus*
			MOLONEYI
C. a. francescae	*C. m. francescae*	*C. m. francescae*	*C. (n.) m. francescae*
C. a. moloneyi	*C. m. moloneyi*	*C. m. moloneyi*	*C. (n.) m. moloneyi*
MITIS	HEYMANSI	HEYMANSI	OPISTHOSTICTUS
C. m. heymansi	*C. m. heymansi*	*C. m. heymansi*	*C. (n.) o. heymansi*
	MITIS	MITIS	
C. m. opisthostictus	*C. m. opisthostictus*	*C. m. opisthostictus*	*C. (n.) o. opisthostictus*
			MITIS
C. m. mitis	*C. m. mitis*	*C. m. mitis*	*C. (n.) m. mitis*
			*C. (n.) m. maesi**
	BOUTOURLINII	BOUTOURLINII	STUHLMANNI
C. m. boutourlinii	*C. m. boutourlinii*	*C. m. boutourlinii*	*C. (n.) s. boutourlinii*
	STUHLMANNI	STUHLMANNI	
C. m. stuhlmanni	*C. m. stuhlmanni*	*C. m. stuhlmanni*	*C. (n.) s. stuhlmanni*
C. m. elgonis	synonym of *stuhlmanni*	synonym of *stuhlmanni*	synonym of *stuhlmanni*
			DOGGETTI
C. m. schoutedeni	*C. m. schoutedeni*	*C. m. schoutedeni*	*C. (n.) d. schoutedeni*
DOGGETTI			
C. doggetti	*C. m. doggetti*	*C. m. doggetti*	*C. (n.) d. doggetti*
KANDTI			
C. kandti	*C. m. kandti*	*C. m. kandti*	*C. (n.) d. kandti*

Figure S.1. Satellite image of Vamizi Island modelling sea level (dashed line) and 10 metre interval contours (solid lines) at the Last Glacial Maximum from a 30 arc second paleo digital elevation raster (Map data: Google, Landsat/Copernicus 2016) (raster data from the climatologies at high resolution for the earth’s land surface areas (CHELSA) project: http://chelsa-climate.org/last-glacial-maximum-climate/; Karger et al. 2017).

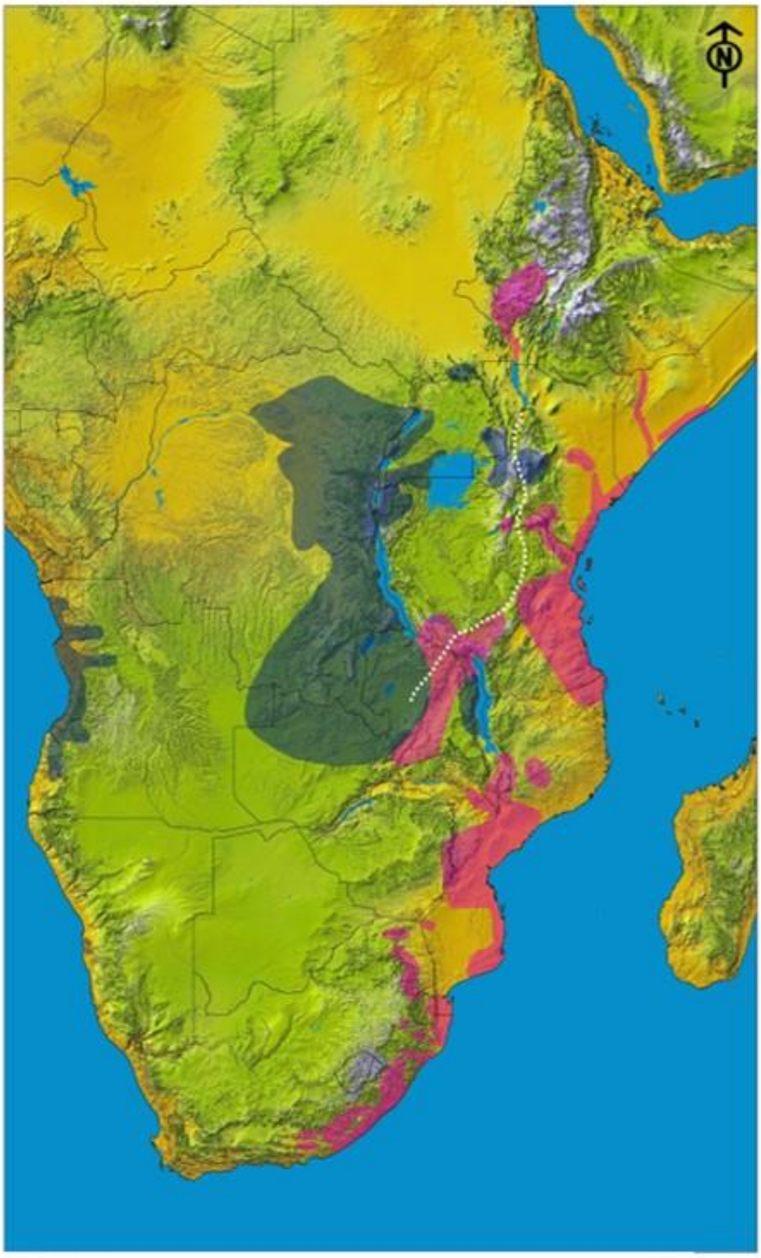

Figure S.2. Proposed distribution of the two species, *Cercopithecus albogularis* (pink) and *Cercopithecus mitis* (blue), based on molecular results in this study and Guschanski et al. (2013). Distribution areas were recreated based on Lawes (2013) map. Subspecies included in *C. mitis* are: *mitis, heymansi* (Colyn and Verheyen 1987)*, francescae, kandti* (Matschie 1905), *opisthostictus* (Sclater 1894)*, stuhlmanni, doggetti* (Pocock 1907)*, schoutedeni* (Schwarz 1928) and *kolbi*. Subspecies included in *C. albogularis* are: *boutourlinii, zammaranoi, albotorquatus, phylax* (Schwarz 1927), *albogularis, monoides, moloneyi, erythrarchus, labiatus* and *"schwarzi"*. Kingdon's line (dashed white line) was recreated based on Joordens et al. (2019) map. Shaded relief image credit: NASA/JPL/NIMA.

Chapter 6: Synthesis: the future of samango monkeys and indigenous forests in the Soutpansberg

1. The impact of habitat fragmentation on samango monkey populations

This study shows that natural and anthropogenic habitat fragmentation have shaped both the distribution (Chapter 2) and the genetic structure (Chapter 3) of samango monkey populations in the Soutpansberg. The distribution of samango monkeys is discontinuous across the mountain range with a major distribution gap in the middle Soutpansberg (Hanglip Forest and east of that), separating populations of the east and west (Chapter 2). Forest patches within the eastern and western survey areas further show limited connectivity with each other due to either geographic features (largely distance) or anthropogenic landscape change (Chapters 2 and 3). This spatial distribution pattern is mirrored by the population genetic results which show overall isolation by distance of populations and lack of contemporary gene flow between them (Chapter 3). Here, particularly the populations at the far eastern and far western ends of the mountain showed signs of heterozygosity loss and potential inbreeding (Chapter 3).

Samango monkeys in our study show a substantial degree of surrounding matrix utilisation indicating that they have behavioural and dietary flexibility to cope, to a certain extent, in this transformed landscape (Chapter 2; Linden et al 2015; Lawes 1990). However, we conclude that although we found samango monkeys frequently using the surrounding matrix, this does not seem to ensure genetic connectivity between populations (Chapters 2, 3 and 4). The matrix utilisation exposes the samango monkeys to a different set of threats than they encounter in their primary forest habitat. Here, we identified road collisions as the biggest direct anthropogenic threat to samango monkeys in the study area.

We found that matrix use differed between maternal groups and lone and bachelor group males with the latter utilising the human dominated matrix more extensively than maternal groups (Chapter 2). While maternal groups only seem to utilise fruit orchards and timber plantations when these were near-adjacent to high canopy, indigenous forests, male samango monkeys were found to range far into transformed areas and were frequently observed in residential gardens (Chapters 2 and 4). We also conclude that timber plantations can play a role in connecting forest fragments although rotational clear felling of plantation stands can result in these corridors abruptly disappearing (Chapter 2).

Another, more natural element of the surrounding matrix is the Soutpansberg Mountain bushveld and here our observations show that this vegetation type is utilised by both lone and bachelor males and maternal groups alike (Chapters 2 and 4). The loss of grasslands and emergence of this vegetation type has led to a significant increase in woody vegetation cover across the southern aspect of the mountain (Hahn 2017). Samango monkeys could be benefiting from increased woody vegetation cover resulting in extension of their home ranges outside of indigenous forests by including such adjacent semi-deciduous woodland habitat (Linden et al. 2015). This localised range extension could enable samango monkeys to inhabit comparatively small forest patches in general and move between such patches (Chapter 2) while also allowing larger groups and/or more groups to inhabit forest patches (personal observation for the Lajuma population in the far western Soutpansberg).

To counteract further loss of genetic diversity in local populations, translocations of lone or bachelor group males from areas where the subspecies *C. a. schwarzi* is known to occur (Chapter 3; Dalton et al. 2015; Linden et al. 2016) could be carefully considered. If populations should experience significant reductions or become locally extinct (as suggested for the Hanglip forest) through random or human induced events, the chance of samango monkeys recolonising forest patches is hindered by distances between these (Chapter 2). As females are highly territorial and philopatric, we are not confident that translocations of individual female group members or entire groups are a viable intervention.

2. *Human-primate conflict and indigenous forest utilisation*

To gain insights into human perceptions of samango monkeys and possible direct anthropogenic pressures on their forest habitat we personally interviewed people who contacted us to report samango monkey sightings and people who lived adjacent to forests inhabited by samangos (Chapter 2) using a standardised set of questions. Here we followed recommendations of White et al. (2005). We only included adults (> 18 years) for the interviews and every person who agreed to be interviewed was informed about what the data will be used for, that their anonymity is guaranteed, and that data will not be handed to a third party. When necessary, translators fluent in both TshiVenda (the predominant local language) and English were used. People were asked questions pertaining to human-primate conflicts (particularly with samango monkeys) and, if any conflict was reported, to specify the nature of the problem and how it was mitigated. We further asked about indigenous forest utilisation including hunting of wildlife, fire wood collection and collection of plants or their parts.

A total of 28 people agreed to be interviewed of which 6 were female and 22 male. Geographically, 10 people were interviewed from the western and 18 from the eastern Soutpansberg. Of the 28 interviewees 18 lived on privately owned land, 6 on community owned land and 4 on state owned land. Land use practices varied from commercial agriculture and silviculture, to subsistence farming, residential, eco-tourism and environmental education.

When asked about conflicts with primates, 68% (n = 19) reported that they had problems with at least one of the three diurnal primate species occurring in the area: chacma baboon (*Papio ursinus*), vervet monkeys (*Chlororcebus pygerythrus*) and samango monkeys (*Cercopithecus albogularis*). In all but one of these cases conflict was in the form of crop raiding (Figure 1). Commercial farmers reported damage to avocado, macadamia and pecan orchards, subsistence farmers suffered damage to mealie fields and vegetable plots and problems in private households were linked to raiding of vegetable gardens, rubbish bins and kitchens. In one case baboons and vervet monkeys were perceived as a threat to birdlife in the garden as they had been observed raiding nests.

The majority (74%) of human-primate conflicts were recorded from residents in the eastern Soutpansberg. Of the 19 people reportedly having conflicts with primates, all but one named vervet monkeys and 12 named baboons. Both species caused conflicts in commercial and subsistence farming areas and in and around houses. Conflicts with samango monkeys were only reported in 4 cases. They were not reported to cause problems for subsistence farmers and none of the commercial farmers considered them a major factor in overall crop damage or loss. Crop raiding by groups of samango monkeys reportedly only occurred in areas where orchards directly border indigenous forests or by single or bachelor group males often associated with groups of vervet monkeys. People frequently reported conflicts with more than one primate species.

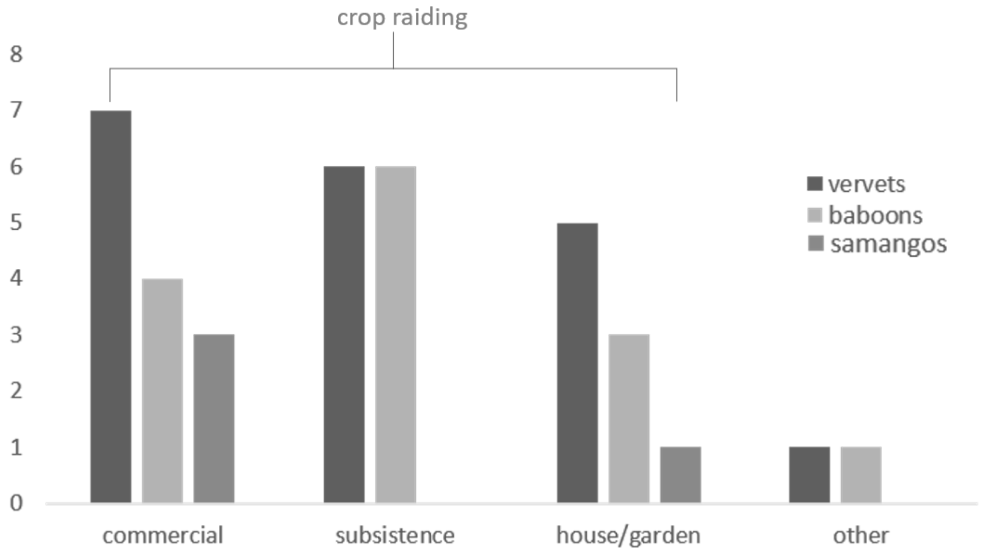

Figure 1. Human-wildlife conflicts with the three diurnal primate species (vervet monkey, chacma baboon, samango monkey). The y-axis shows the number of interviewees reporting conflicts with any of the three primate species. Conflicts are divided into ‘crop raiding’ in commercial and subsistence farming areas and in and around residential houses/gardens and ‘other’ (non-crop raiding).

When asked about how conflicts were being mitigated eight different strategies were reported (Figure 2): shooting (with a rifle to kill primates), chasing (by people), fire crackers (to scare primates away), dogs (to chase or kill primates), waste management (around houses and gardens to prevent primate access), cage traps (to catch and then kill primates), poison (fruits laced with Aldicarb (Temik) to kill primates) and catapults (to scare primates away). People generally used several different mitigation measures. Use of one of the four mitigation measures aimed at killing primates (shooting, dogs, trapping, poison) was reported by 65% (n = 13) of the interviewees.

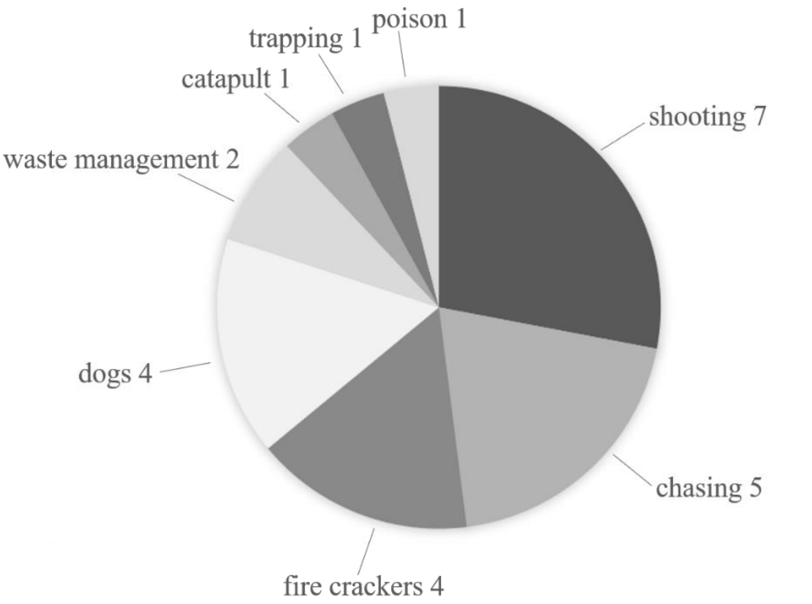

Figure 2. Number of interviewees using the various mitigation strategies to manage conflicts with primates.

When looking at the mitigation measures used for each primate species, none of the methods aimed at killing primates are used for the samango monkey (Figure 3). Two commercial farmers stated that they do not use any mitigation measure if they see samango monkeys in their orchards as this is very infrequent. When asked about the general attitude towards the presence of samango monkeys in their areas, every interviewee gave a positive response. Vervet monkeys are seemingly under the highest persecution pressure as they reportedly cause the greatest damage to both subsistence and commercial farmers.

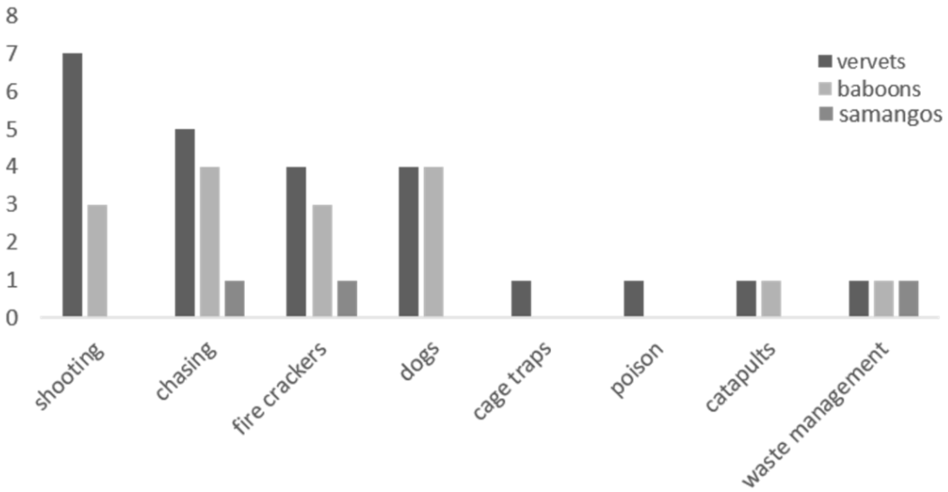

Figure 3. Number of people reporting use of the various conflict mitigation measures for the three diurnal primate species (vervet monkey, chacma baboon, samango monkey) across the Soutpansberg.

Regarding the utilisation of forest and wildlife products 50% (n = 14) of interviewees reported knowing about wildlife being hunted in forests. Here, most cases (79%, n = 11) occurred in the eastern Soutpansberg. Hunting occurred using snares (n = 14) and dogs (n = 12). Dogs are reportedly used to either directly kill the animal or for baying it so that the hunter can bring it down with spears or axes. Species targeted include bush pig (*Potamochoerus larvatus*), red duiker (*Cephalophus natalensis*), grey duiker (*Sylvicapra grimmia*) and bush buck (*Tragelaphus scriptus*). The collection of plants or their parts was reported by 39% (n = 11) of people interviewed with most reports (n = 9) from the eastern Soutpansberg. Lastly, the collection of fire wood was reported by 32% (n = 9), all from residents of the eastern Soutpansberg.

We can conclude that of the three diurnal primate species found in the Soutpansberg, samango monkeys do not cause large scale damage to farmers and are not currently considered a major problem. However, as agricultural land continues to expand and encroach on indigenous forests (Chapter 2; Munyati & Kabanda 2008) samango monkeys might become more of a problem to farmers in future. This should be researched and monitored. Baboons and particularly vervet monkeys are clearly subjected to a high level of direct persecution as they cause large scale crop damage and loss which is likely linked to them being more terrestrial and less confined to closed canopy forest habitat. Again, we found clear differences between the eastern and western Soutpansberg in terms of conflicts with primates and utilisation of forest resources.

3. *Climate change impacts on forests*

When assessing samango monkey persistence in the Soutpansberg, possible future climate change impacts on forest habitat must be considered (Chapter 2). While average global temperatures are predicted to rise by up to 2.5 °C by 2050 (IPCC 2013), trends in Africa suggest the continent may experience double this rate of change (Engelbrecht et al. 2016) with tropical southern Africa being one of the fastest warming areas (Davis-Reddy & Vincent 2017). Over the last century average temperatures across greater southern Africa have shown a clear and marked increase (Kruger & Nxumalo 2017) and future temperature projections show rises of 2–4 °C in the interior (Davis-Reddy & Vincent 2017). These mean temperature increases are further accompanied by increases in extreme events such as drought, heavy rainfall events, strong winds and wildfires (Davis-Reddy & Vincent 2017). Rainfall predictions for southern Africa are ambiguous with disagreement on wetting vs. drying trends (Skowno et al. 2019) but in the north-eastern parts of South Africa, where the Soutpansberg is located, trends indicate that rainfall will reduce, and average annual temperatures and atmospheric CO_2 levels will continue to rise (Kruger & Sekele 2013; DEA 2013; Engelbrecht et al. 2016).

The potential impact of these changes on forests is diverse and temperature changes alone are likely to drastically alter forest ecosystem dynamics (Norby et al. 2007). Higher temperatures lead to elevated water losses from evaporation and evapotranspiration and can reduce water use efficiency of plants (Mortsch 2006). Moisture stress and drought can lead to increased susceptibility to disturbances such as insect pests, pathogens and forest fires (Moore & Allard 2008). Temperature and precipitation changes can further make forests more sensitive to alien plant invasion (Dale et al. 2001). Increased CO_2 levels generally promote plant growth but only as long as other factors such as water do not become limiting (Moore & Allard 2008). A consistent response of plant species to climate change entails changes in distribution and here forests plants are expected to shift their ranges northwards or to higher altitudes with warming temperatures (Parmesan 2006; Schreiter et al. 2017).

Taking all these impacts together we suggest that indigenous forests of the Soutpansberg are very vulnerable to climate change in future. Many forest patches and riverine corridors are naturally elongated and narrow with high perimeter: area ratio (Chapter 2), making them naturally vulnerable to edge effects such as changes in temperature, humidity and amount of sunlight penetrating, increasing risks of fires and alien invasion (Primack 1993). Forests in the Soutpansberg are driven by shade effects and runoff from cliffs, rainfall, fog precipitation and groundwater. The predicted drying trend and increased mean temperatures for the region might result in forest tree die-offs, plant community changes, overall forest cover shrinkage (Rutherford et al. 1999) and increased susceptibility to disturbances such as invasion by already-present (Agricultural Research Council; www.arc.agric.za) alien invasive plants (for example Mauritius thorn (*Caesalpinia decapetala*), black wattle (*Acacia mearnsii*), blue gum (*Eucalyptus* spp.) and bug weed (*Solanum mauritianum*)). As most forests in the Soutpansberg are probably already at their altitudinal range limits and confined to very specific localities such as ravines or below south facing ridges, a distributional shift of forests to higher altitudes is not considered a realistic scenario (Chapter 2).

Soutpansberg Mountain Bushveld has already been described to benefit from the increased atmospheric CO_2 levels (Hahn 2017) and as it is comprised of woody species that are more drought tolerant than broadleaf evergreen trees, climate change might not impact on this vegetation type as profoundly. Although samango monkeys have been shown to utilise this vegetation type as corridors and for foraging (Chapter 2; Linden et al. 2015) and thus seem to benefit from the increased woody vegetation cover adjacent to primary forest habitat (as opposed to the historic open grasslands), they still rely on the presence of high canopy, evergreen forest (Chapter 2).

4. Phylogeny of African forest vertebrates

Phylogenetic studies play an important role in identifying evolutionary patterns and processes, allowing for reconstructing migration and colonisation patterns (phylogeography), tracing ancestral relationships between species and unveiling cryptic species thus directly informing taxonomic classifications (Soltis & Soltis 2003; Sereno 2005; Sinclair et al. 2005). Incorrect taxonomic identification and lack of knowledge of a species genetic and geographic history can hamper successful conservation efforts (Sinclair et al. 2005; Bortolus 2008).

Phylogenetic patterns found for samango monkeys (Dalton et al. 2015; Chapter 5) resemble patterns found for other forest dwelling species which underwent the same repeated forest habitat expansion and contraction due to oscillating glacial and interglacial periods in the Pliocene and Pleistocene in east and southern Africa. Specifically, the Plio-Pleistocene transition (3.6-2.4 MYA) and the Mid-Pleistocene transition (1.25-0.7 MYA) played important roles in shaping genetic structure and diversity in vertebrate populations including forest dwelling taxa (Mayr & O'Hara 1986; Bibi & Kiessling 2015). Owing to more recent phylogenetic studies, it is now suggested that Mayr and O'Hara's (1986) forest refugia hypothesis may not only help to explain speciation patterns of the Pleistocene but also of the late Pliocene as found in a study on African forest robins (Voelker et al. 2010). Our phylogenetic results in Chapter 5 also indicate that Pliocene forest refugia may have played a role in the divergence of *C. mitis* and *C. albogularis* (2.57 MYA). This together with the general patterns of distinct geographical associations of these two clades (largely Afromontane and central African lowland forest west of the Eastern Rift Valley in *mitis* and largely Afromontane and coastal forests east of the Eastern Rift Valley in *albogularis*, extending to southern Africa) we find compelling evidence for recognizing these two clades as valid evolutionary species, as proposed by Groves (2001). The time of divergence of *mitis* and *albogularis* in central-east Africa occurred during the Plio-Pleistocene transition, a climatic period of change from warm Pliocene climates to Pleistocene ice ages characterised by the intensification of Northern Hemisphere Glaciation (iNHG) from 3.6 -2.4 MYA (Mudelsee & Raymo 2005). In addition to climatic oscillation, complex tectonic processes in East Africa acted as another driver of forest fragmentation from the mid-Miocene onwards (e.g. Ring et al. 2018). For forest dwelling species this habitat fragmentation would have caused a similar scenario as described for the mid-Pleistocene period, that of isolated populations in forest refugia, favouring speciation. It is interesting to note here that forest cobras (*Naja subfulva*) show a distribution pattern reminiscent to that of *mitis/albogularis*, having radiated out of central Africa down into lowland forests of South Africa (largely found in coastal forests of northern Kwa-Zulu Natal) (Wüster et al. 2018).

Increased intra and interspecific diversification in forest taxa in the mid-Pleistocene was for example found in forest duikers (Cephalophinae) (Johnston & Anthony 2012) and starred robins (*Pogonocichla stellate*) (Bowie et al. 2006) and was often accompanied by latitudinal range expansions from east into southern Africa as we describe for the samango monkey in Chapter 5. Other forest vertebrates showing this pattern are forest shrews (for the genus *Myosorex*) (Taylor et al. 2013), miniature leaf litter frogs (*Arthroleptis xenodactyloides*) (Blackburn & Measey 2009) and olive sunbirds (*Nectarinia olivacea/obscura*) (Bowie et al. 2004).

Forest dwelling vertebrate species found in southern Africa today are the result of resilient ancestral lineages able to adapt and persist through the long history of repeated natural forest fragmentation. This can also reflect in lower levels of genetic diversity of forest species found at the southern range limits compared to species in large, contiguous tropical lowland forests as discussed in Chapter 5. Even on a smaller scale in South African forests, Lawes (2007) could show that extinction filtering (Balmford 1996) caused by paleoclimatic changes shaped vertebrate communities in the countries three main forest types. Here, forest species diversity and composition varying markedly between them with the greatest forest faunal diversity found in scarp and coastal forests in northern Kwa-Zulu Natal. Afromontane forests in South Africa are comparatively species poor and appear most affected by climatic extinction filtering.

5. *Future research and conservation*

Results from this study lead us to recommend that conservation and management strategies aimed at the long-term persistence of the samango monkey populations and their forest habitat across the Soutpansberg should be formulated separately for the eastern and western parts of the mountain range as differing threats and landscape variables need different conservation and management approaches. We further call for explicit mention of different management requirements for groups and lone or bachelor males to achieve a holistic conservation approach for samango monkey populations on the landscape scale (see below). With road fatalities being the most important direct threat to samango monkeys we recommend continued future research and efforts to mitigate this issue.

5.1. Recommendations for the western Soutpansberg

Forests in the western Soutpansberg are generally surrounded by a natural (but in many cases novel; Hahn 2017) matrix composed of Soutpansberg Mountain Bushveld with very little landscape transformation for commercial agriculture or silviculture (Chapter 2). Here we did not identify any direct anthropogenic threats to samango monkeys that would be of great concern. We recorded two incidences, one of a male samango monkey which was part of a bachelor group of two who was killed by a domestic dog in a farmhouse garden and one which was electrocuted when using a power line (pers. obs.). However, as the human population density and infrastructure is very low in the western parts of the mountain with large areas of privately-owned land we do not consider these isolated incidents to be a major threat to the samango monkey population. Both the overall forest cover and individual forest patch size are smaller in the west (typically < 100 ha per patch) compared to the east. The largest forest patch in the western Soutpansberg (Hanglip Forest, 234 ha) was found unoccupied by samango monkeys (Chapter 2). In the west, samango monkeys were found to occupy at least 48% of suitable forest habitat. Forest connectivity in the west is largely hampered by geographic distance causing populations to be isolated from each other, both spatially and genetically

(Chapters 2 and 3). Here the population in the far west is of most conservation concern as it shows the lowest heterozygosity and connectivity among all populations (Chapter 3).

Interview data showed that only one person in the western Soutpansberg had a problem with samango monkeys raiding food in and around houses but that this problem was controllable through strict food and waste management and education of tourists. Overall, people had a positive attitude towards the presence of samango monkeys. Direct utilisation of forest products was also limited and reported incidents were infrequent and isolated, indicating that direct anthropogenic pressure on indigenous forests in the western Soutpansberg is comparatively low.

In the west 41% of samango monkey's area of occupancy is under formal protection, including the privately owned Luvhondo Nature Reserve which we consider one of the most important areas for samango monkeys as it contains the largest occupied contiguous forest, a stronghold for samango monkeys in the far-western Soutpansberg (Chapter 2).

We conclude that climate change, changing land ownership and further loss of genetic diversity due to limited connectivity are the main threats to samango monkey populations in the western Soutpansberg.

The most important conservation and management recommendation is to ensure the continued protection of forest habitat and to preserve a corridor between the two existing protected areas of Luvhondo and Happy Rest. As the majority of western samango populations inhabit forests on private land (Chapter 2), we consider landowner engagement important for creating awareness about indigenous forest and samango monkey conservation as changing landownership could impact negatively on the protection of both.

5.2. Recommendations for the eastern Soutpansberg

The landscape of the eastern Soutpansberg is dominated by human activities creating a very diverse matrix surrounding remaining forest patches (Chapters 2, 3 and 4). The landscape is characterised by a comparatively high human population density resulting in heavy land use and landscape change due to commercial and subsistence agriculture, extensive silviculture, urban expansion with rural communities often still directly reliant on natural resources such as fire wood and a much denser road and electricity network. Enveloped in this are remaining indigenous forest patches supporting populations of samango monkeys. The eastern Soutpansberg contains the largest forest area in the mountain range with the samango monkeys occupying the majority (78%) of it. However, only 15% of forests are under formal protection in the east.

The samangos showed a substantial degree of matrix utilisation including fruit orchards, timber plantations, human residences and crossing a main provincial paved road (Chapter 2) and road fatalities were recorded regularly in the matrix, a direct threat which needs suitable mitigation measures to avoid local population reduction and ensure genetic connectivity (Chapter 4). Localities of roadkills indicated that the samango monkeys also utilise timber plantations to a certain extent to navigate through the matrix (Chapter 2). These plantations could offer important corridors but are subject to regular clear-felling.

We detected a difference in the degree of matrix use between maternal groups and lone or bachelor males in the east. Males were shown to travel much greater distances through the matrix away from primary forest habitat and were often found in residential gardens where they were often reported to spend several months at a time (Chapter 2). Despite males being able to travel some distance outside of primary forest habitat, our population genetic data show that the population in the far east seems to be more isolated than would be expected by distance suggesting that anthropogenic landscape change hampers dispersal between forests (Chapter 3). It is apparent that riverine forests are of importance in this highly transformed landscape as they act as corridors between high canopy forest patches.

Ongoing deforestation and thus further habitat loss and fragmentation (Chapter 2; Munyati & Kabanda 2008) are considered a main threat to forests in the eastern Soutpansberg and one of the most important recommendations for the eastern Soutpansberg is improved indigenous forest habitat management. Our interview data showed that forests and their fauna are under much more direct anthropogenic pressure in the east compared to the west and we advocate for much stricter law enforcement to be implemented. A main conservation focus should be given to the three large forest fragments, Luonde, Entabeni and Thathe Vondo, as we consider them the stronghold for samango monkeys in the east. In order to facilitate connectivity between these areas, small forest patches within the human dominated matrix between them, riverine forests and even certain areas of timber plantations should be managed and protected as corridors.

5.3. Mitigation measures: future and challenges

The next step towards roadkill mitigation for samango monkeys at actual roadkill sites requires the building of a prototype bridge according to specifications prescribed by the South African National Roads Agency Limited (SANRAL). This should ideally be done through the inclusion of engineers and road developers to ensure a sound design suitable for public roads. Again, this prototype could then be erected at the Lajuma Research Centre where it can be monitored if samango monkeys and other arboreal species use the structure. Constructing a prototype would also enable us to estimate costs involved and form the basis of a cost-benefit analysis which can serve as an economic argument why the installation of crossing structures could be of value (Huijser et al. 2009). It is estimated that R 1.3 billion are spent annually on accident insurance claims possibly attributed to wildlife-vehicle collisions in South Africa (Collinson et al 2015). Health insurance claims and private medical, or at worst, funeral costs can also be encountered. Taking this into account, it becomes clear that the construction of wildlife crossing structures would not only benefit biodiversity conservation but also public health and safety. The argument of public health and safety also promotes that decision making on putting up crossing structures should not just focus on weather target species are rare and endangered as many common and often sizable mammal species (e.g. kudu) are involved in vehicle collisions. Certainly, as is the case with the samango monkey, rare and endangered species can act as umbrellas and be used for public awareness and to gain public support for the installation of wildlife crossing structures. However, crossing structures should ideally be designed to create habitat connectivity for a variety of species while reducing health and safety risks for humans.

There are over 40 different types of wildlife crossing structures used globally to mitigate roadkill, including underpasses and overpasses (with and without fencing) as well as measures focusing on altering animal (e.g. scaring animals away from roads) or driver behaviour (e.g. speed limits, road signs) (Rytwinski et al., 2016). Deciding on the correct structure can however be challenging as there is often comparatively little information on the effectiveness of interventions to reduce roadkill. A metanalysis of studies across the globe could show, that mitigations reduce roadkill by 40% with crossing structures that include fencing being one of the most effective interventions (Rytwinski et al. 2016). It is recommended that the decision on the correct locality for installing crossing structures should be based on a robust, long term (four year) spatial dataset.

Compared to developed nations in Europe or North America where large numbers of interventions have been constructed and tested for almost 40 years, South Africa is still in its infancy when it comes to the implementation of roadkill mitigation measures on its road network (Collinson et al. 2019). One major reason for this can be the lack of resources and the allocation of available resources towards more pressing socio-economic issues (Collinson et al. 2015). For example, South Africa has a very high unemployment rate (29.1%), the biggest HIV epidemic in the world (20.4%) and over half of all South African's are living in poverty (55.5%) (Statistics South Africa 2015; 2018; 2019). There is certainly still a need to improve engagement with road developers and SANRAL to ensure that research findings on the roadkill issue are shared and translated more directly to create a far greater awareness of the problem. As shown in developed nations, a constant dialogue between all interested and effected parties is paramount for successful roadkill mitigation planning and implementation. Applying our findings from Chapter 4 to real conservation action on the ground will in all reality not be a fast or easy process and require a lot of leg-work and convincing. One strength of the South African road ecology work to date is that a lot of spatial and behavioural data collection has been undertaken providing a sound basis for advising on suitable structures and localities, monitoring schemes and possible impact. Developing countries often have the reverse situation in that they have resources needing to be spend without enough background research available to ensure successful mitigation. In several cases this has resulted in expensive crossing structures placed at wrong localities or in crossing structures not used by wildlife as was imagined (Collinson pers. comm.).

Lastly, we consider awareness campaigns essential in the process to ensure public support. South Africa's biggest conservation organisation, the Endangered Wildlife Trust (EWT), has an entire programme dedicated to road ecology (including research and public engagement) with which we have established a close partnership for the samango monkey roadkill mitigation project.

6. Overall conclusion

The phylogeographic research we undertook gives valuable insights into the species evolutionary history showing that these monkeys are very adaptable persisting in highly fragmented forest habitat in much of southern Africa, having undergone major range expansions and contractions since the Pleistocene when they radiated into the region (Chapter 5; Lawes 1990; Dalton et al. 2015). Here they adapted to several different forest types (coastal dune forests, scarp forests, Afromontane forests) some of which are quite marginal (e.g. the Malangeni swamp forest in Kozi Bay or the scrubby, thicket like vegetation on the island of Bazaruto in Mozambique) (Lawes 1990). However, the time frames involved were much longer than changes happening today due to human expansion for agriculture and living space. Going into the future, it remains to be seen how these fairly matrix tolerant (Chapter 2) monkeys will be able to survive under the many pressures they and their forest habitat are challenged with. Protecting the remaining forests in the Soutpansberg and managing corridors is paramount for the samango monkey's long-term survival but to what extent climate change will impact on forests and if this can be mitigated in any way is not certain.

However, as the attitude of people towards the samango monkey was generally very positive they would make a suitable ambassador for indigenous forest protection. Conservation plans aimed at protecting samango monkeys would benefit many other forest plant and animal species making them a good example, acting as both a flagship and an umbrella species (Simberloff 1998; Caro & O'Doherty 1999). Findings of our study are not only relevant for samango monkey populations in the Soutpansberg but also for populations elsewhere in South Africa and southern Africa where forests and samango monkeys share the same paleohistory and current anthropogenic threats.

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Appendices

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RESEARCH ARTICLE

New Insights into Samango Monkey Speciation in South Africa

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Abstract

The samango monkey is South Africa's only exclusively forest dwelling primate and represents the southernmost extent of the range of arboreal guenons in Africa. The main threats to South Africa's forests and thus to the samango are linked to increasing land-use pressure and increasing demands for forest resources, resulting in deforestation, degradation and further fragmentation of irreplaceable habitats. The species belongs to the highly polytypic *Cercopithecus nictitans* group which is sometimes divided into two species *C. mitis* and *C. albogularis*. The number of subspecies of *C. albogularis* is also under debate and is based only on differences in pelage colouration and thus far no genetic research has been undertaken on South African samango monkey populations. In this study we aim to further clarify the number of samango monkey subspecies, as well as their respective distributions in South Africa by combining molecular, morphometric and pelage data. Overall, our study provides the most comprehensive view to date into the taxonomic description of samango monkeys in South Africa. Our data supports the identification of three distinct genetic entities namely; *C. a. labiatus*, *C. a. erythrarchus and C. a. schwarzi* and argues for separate conservation management of the distinct genetic entities defined by this study.

Introduction

The geographical distribution of the arboreal guenon *Cercopithecus albogularis* ranges from central and eastern to southern Africa where it occurs in different evergreen forest types including rainforest, Afromontane and riparian forests, as well as swamp and coastal forests [1]. The species belongs to the highly polytypic *Cercopithecus nictitans* group [2] which is sometimes divided into two species *C. mitis* and *C. albogularis*. Groves [3,4] recognises both species and uses the classification of *C. albogularis* for individuals distributed from Ethiopia to South

Appendix A.1. Research article on samango monkey speciation.

Cercopithecus albogularis – Samango Monkey

JL Hilton

Regional Red List status (2016)	
C. a. labiatus	**Vulnerable B2ab(ii,iii,v)***
C. a. erythrarchus	**Near Threatened B2ab(ii,iii,v)***
C. a. schwarzi	**Endangered B2ab(ii,iii,v)***
National Red List status (2004)	
C. a. labiatus	Endangered B1ab(ii,iii,iv,v)
C. a. erythrarchus	Vulnerable B1ab(i,ii,iii)+2ab(i,ii,iii)
C. a. schwarzi	Not Evaluated
Reasons for change	
C. a. labiatus	Non-genuine: New information
C. a. erythrarchus	Non-genuine: New information
C. a. schwarzi	Non-genuine: Taxonomy
Global Red List status (2008)	
C. a. labiatus	Vulnerable A2c
C. a. erythrarchus	Least Concern
C. a. schwarzi	Not assessed
TOPS listing (NEMBA)(2007)	Vulnerable
CITES listing (1977)	Appendix II (species level)
Endemic	
C. a. labiatus	Yes
C. a. erythrarchus	No
C. a. schwarzi	Unknown

*Watch-list Data

The Zulu word for Samango Monkey, *insimango*, means "monkeys in the mist".

Taxonomy

Cercopithecus albogularis labiatus Geoffroy Saint-Hilaire 1842

Cercopithecus albogularis erythrarchus Peters 1852

Cercopithecus albogularis schwarzi Roberts 1931

ANIMALIA - CHORDATA - MAMMALIA - PRIMATES - CERCOPITHECIDAE - *Cercopithecus* - *albogularis*

Synonyms: *Cercopithecus mitis* Geoffroy Saint-Hilaire 1842

Common names: Samango Monkey, Stair's White-collared Monkey, Schwarz's White-collared Monkey (English), Samango-aap (Afrikaans), Insimango (siSwati, Zulu), Ndlandlama (Tsonga), Dulu (Venda), Intsimango (Xhosa)

Taxonomic status: Subspecies

Taxonomic notes: There are taxonomic controversies on both the species and subspecies level. While Groves (2001, 2005) classifies the Samango Monkey as *C. albogularis* (recognising *albogularis* as a separate species within the highly polytypic *Cercopithecus nictitans* group), Grubb et al. (2003) do not recognise *C. albogularis* as a separate taxon and classify it as *C. mitis*. No genetic analysis has been done to date to support one or the other classification. The number of subspecies recognised in South Africa is also inconsistent. Meester et al. (1986) followed by Grubb et al. (2003) recognise two subspecies, namely *C. a. labiatus* and *C. a. erythrarchus*, whereas Roberts (1951) followed by Dandelot (1974) and (Groves 2001) recognise an additional third Samango Monkey subspecies in South Africa, namely *C. a. schwarzi* Roberts 1931. Recently, Dalton et al. (2015) found clear pelage colour polymorphism in South African Samango Monkeys and were able to identify three distinct geographical colour morphs as well as three genetically distinct entities: Hogsback (Eastern Cape Province) corresponding to *C. a. labiatus*, Inland (Soutpansberg, Magoebaskloof) corresponding to *C. a. schwarzi*, and Coast (Cape Vidal, Sodwana Bay) corresponding to *C. a. erythrarchus*. These findings support the presence of three subspecies within the assessment region, as first proposed by Roberts (1951) and as currently accepted by Groves (2001).

Assessment Rationale

Samango Monkeys are restricted to a variety of forest habitats and comprise three subspecies within the assessment region: Samango Monkey (*C. a. labiatus*), Stair's White-collared monkey (*C. a. erythrarchus*), and Schwarz's White-collared Monkey (*C. a. schwarzi*). While *C. a. labiatus* is endemic to the assessment region, *C. a. erythrarchus* occurs throughout southern Africa and it is uncertain whether *C. a. schwarzi* is endemic due to lack of sampling in suitable extra-regional habitats. However, according to current data the latter subspecies is, at this

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Appendix A.2. Samango monkey Red List assessment.

LIMPOPO MIRROR

Nature Conservation

Do you know where the monkeys stay?

Residents of the Soutpansberg are urged to assist researchers in a survey to determine the distribution of Samango Monkeys in die mountain range.

"What is urgently needed is a proper assessment of Samango distribution and population status in order to help the conservation and management of these monkeys. As part of my doctoral research, I am currently establishing in which forest patches in the Soutpansberg Samango Monkeys occur," said Mrs Bibi Linden. She added that Samangos had also been unofficially recorded in the Blouberg mountain range. Samango Monkeys are found only in and near high-canopy forests, which, in the Soutpansberg, are found mostly along the northern slopes. In this they differ from the widely distributed Vervet Monkey, which inhabits much drier habitat such as acacia woodland.

Samango Monkeys also differ in size and appearance from Vervet Monkeys (see pictures). As the Samango needs a high-canopy forest habitat, its distribution is very restricted and patchy across South Africa. Forests, and thus all the species inhabiting them, are under enormous pressure (directly bordered by timber plantations, suffering from deforestation and invasion by alien plants, etc.). Samango Monkeys are listed as vulnerable in the Red Data Book of the Mammals of South Africa.

"The first step of my project involves generating a list of Samango Monkey sightings across the study area. As the study area is large, I can not survey every forest patch in the Soutpansberg for the presence or absence of Samango Monkeys and am thus asking the public for help in this regard," says Linden. She requests everyone who has seen Samango Monkeys or has them on their farm to contact her. If possible, the date and time of sighting, number of individuals encountered, name of forest patch or farm and GPS coordinates must be provided.

For more information or to report a sighting, contact Linden at 071 105 8117 or email at birthelinden@gmx.de

Vervet Monkeys has a black face with legs the same colour as the body. Males have a bright blue scrotum. *Photo supplied.*

The Samango Monkey has a light grey to grey face, black front legs, grey hind legs, orange coloured fur at the tail base (sometimes even a slight orange shimmer on the back), very long and fluffy hair on the cheeks and is generally larger in body size and has longer, shaggier fur compared to Vervet Monkeys. *Photos supplied.*

Nature conservation

"Is this a male or a female monkey?"

As part of her PhD research into samango monkeys, Ms Bibi Linden says she has received great support from residents all over the Soutpansberg.

"In my research on samango monkeys, I have been lucky enough to meet many generous people who have reported samango monkeys in their area or even in their gardens. People are often visited by only one or two samangos and an important question I ask is whether they are males or females," says Linden.

Linden explains that samango females remain in the area in which they were born for their entire life and are assumed never to move far away or even outside of high-canopy forests.

"Samango troops have only one adult male and the core of samango societies is formed by related adult females and their offspring. Male samango monkeys, however, are forced out of their birth troop as soon as they reach sexual maturity. These young males then disperse to find a new troop of females and overthrow the resident troop male, who then has to retire elsewhere," says Linden.

The reason why the question of gender is important, says Linden, is that she needs to know if people are coming across females exhibiting unusual behaviour or if these are young dispersing/old retiring males.

In order to identify the gender of a samango monkey, it is best to use binoculars but if these are not available their calls can give certainty.

A rough guide to identifying the gender of a samango monkey:

* Male samango monkeys are much larger than females (males 8 to 10kg, females 4 to 5kg);

* Male samango monkeys have large canines (see picture);

* Only male samango monkeys give a distinct alarm call (loud "pyow", or repeated "kwa");

* Females have two visible nipples protruding through the fur (see picture); and

* Males parts are best visible when the animal is sitting or from behind/underneath (see picture).

For more information on samango monkeys and sightings, contact Linden at Tel 071 105 8117, or by email at birthelinden@gmx.de or birthe.linden@univen.ac.za

Male samango monkeys. *(Photos supplied).*

Female samango monkey. *(Photos supplied)*

Appendix A.3. Calls for public participation in collecting samango monkey distribution data through local newspapers.

ZOUTPANSBERGER

Conservation

Please brake for these vulnerable monkeys

In an effort to make residents more aware of Samango monkeys in the Soutpansberg, residents of Makhado (Louis Trichardt) can now buy an I BRAKE FOR SAMANGO MONKEYS sticker.

As part of her PhD research into Samango monkeys, Mrs Bibi Linden says she has received great support from residents all over the Soutpansberg.

"Recent reports also included two Samango monkey road kills on the Thohoyandou road," Linden says. She added that reports indicate that these monkeys seem to be crossing this road regularly on a 30km-long stretch between the Piesanghoek turn-off and the second Levubu turn-off further east.

"It was also reported to me that Samangos infrequently cross the Vivo road in the western part of the Soutpansberg. In response to this, and based on the fact that Samango monkeys are nationally listed as 'vulnerable' and are considered a rare species, I started a local awareness campaign as part of my research," Linden says.

As part of the campaign, Leach Printers sponsored two road signs (which will be mounted on the Thohoyandou road, warning drivers about the crossing of Samangos) and bumper stickers with the slogan "I BRAKE FOR SAMANGO MONKEYS".

"I am asking local people to support Samango monkey conservation actively by purchasing a Samango monkey bumper sticker. By putting stickers on the back of a car, people are making a public statement and are also educating fellow drivers," Linden says.

Stickers can be purchased directly from Linden or at OK Grocer at the tobacco counter for a nominal fee of R2. Revenues and donations will be used for continuing and growing the Samango monkey awareness campaign.

"It is important for me to collect information about where exactly Samangos are crossing roads to focus conservation efforts in those areas. In this regard, I appreciate any reports of where exactly people see Samangos crossing or see dead Samangos on a road. Although a very sad incidence, a Samango killed by a car allows me to collect a variety of data, such as tissue samples for DNA analysis," Linden says. Samango sightings can be reported to Linden at Tel 071 105 8117, or by email at birthelinden@gmx.de or birthe.linden@univen.ac.za

Mr Andy Leach (right) from Leach Printers and Mrs Bibi Linden (left) in front of one of the sponsored sign posts. ***Photo supplied.***

Appendix A.4. Awareness campaign for samango monkey roadkill mitigation using public newspapers.

[redacted] [redacted] [redacted] [redacted] [redacted] [redacted] [redacted]

Appendix A.5. Bumper stickers for the samango monkey roadkill mitigation awareness campaign.