Evolutionary History and Conservation of the Endangered Sanje Mangabey (*Cercocebus sanjei*) in the Udzungwa Mountains, Tanzania

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SUMMARY

The Sanje mangabey (*Cercocebus sanjei*) is a primate species that is endemic to the Udzungwa Mountains, Tanzania. The species is classified as Endangered due to its putatively declining population size, limited habitat extent, and habitat fragmentation. The species is divided into two populations: one isolated to the Mwanihana forest fragment in the Udzungwa Mountains National Park, and one to the Uzungwa Scarp Nature Reserve forest. The population in Mwanihana is well-protected under National Park regulations, however the Uzungwa Scarp is considerably lesser protected under Nature Reserve regulations. The Sanje mangabey was described in 1979 and studies have been conducted to increase our understanding of the species since, but many knowledge gaps remain. This thesis aimed to address the priority gaps in our understanding to inform conservation action planning. This included conducting the first systematic population survey for the species, and to investigate the genetic diversity and structure of the two populations. Additionally, it has been previously argued whether the Sanje mangabey should be considered at species or subspecies (*C. galeritus sanjei*) level due to its behavioural similarities to other central/east African *Cercocebus* mangabeys. Firstly, we developed a novel acoustic survey method for systematically and more effectively surveying the Sanje mangabey. We estimated the total population size to be 3,167 individuals (95% CI: 2,181-4,596) and found a significantly lower group density in the Uzungwa Scarp forest than Mwanihana. Secondly, we estimated the phylogenetic history of the species and unexpectedly found the Sanje mangabey to be an evolutionarily distinct lineage from other *Cercocebus* mangabeys, diverging 2.17 MYA. Further unexpected results from this study was the designation of the two populations as evolutionarily significant units, having diverged 0.71 MYA. Thirdly, we investigated the phylogeographic structure of the two populations using mitochondrial DNA and found the populations to be significantly differentiated. Further, an ecological niche model combined with a study of the demographic history of each population revealed the populations to have remained stable in recent history, reflective of the environmental stability of the montane forests in the Udzungwa Mountains. Finally, combining life history data with estimated threats to viability for the Sanje mangabey, we estimated loss of habitat to be the greatest threat to each population and therefore conservation recommendations were made based on these results. The results of this thesis will be used to inform conservation management development for the Sanje mangabey within the Mangadrill Conservation Action Plan, with priority placed on the need to increase protection of the evolutionarily significant population in the Uzungwa Scarp forest.

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TABLE OF CONTENTS

TABLE OF CONTENTS: TABLES

TABLE OF CONTENTS: FIGURE LEGENDS

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Go well.

Cheers

CHAPTER ONE

General Introduction

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General Introduction

Non-human primates (hereafter, primates) are currently facing an extinction crisis, with an estimated 60% listed as threatened by extinction and an estimated 75% with a declining population size (Estrada *et al.* 2017). The major conservation threats to primates have been associated with the rapidly increasing human population since the 1950's leading to a greater and unsustainable demand on natural resources, resulting in habitat loss and degradation. Between 2001-2017, forest loss in the four regions in which primates are found; Neotropics, Africa, south Asia and southeast Asia, was an estimated 178.8 million ha at a rate of 10.52 million ha/year (Estrada *et al.* 2019). An estimated 76% of primate species are threatened by habitat loss for agriculture, 60% for logging and 31% for livestock farming, an estimated 60% are threatened by hunting (including for bushmeat, pet trade and conflict), and 2-13% threatened by other habitat altering activities such as road/rail construction, drilling for oil and gas, and mining (Estrada *et al.* 2017).

1.1. Sanje mangabey (*Cercocebus sanjei*)

The Sanje mangabey (*Cercocebus sanjei*; Figure 1.1) is a primate species endemic to the Udzungwa Mountains, south-central Tanzania. They were first described in 1979 by Homewood & Rodgers (1981). The mangabey is a medium-sized primate with a long tail, fawn-coloured pelage, pale pinkish face, grey muzzle and eyelids lighter than the colour of the face. Following results from long-term primate surveys throughout all forest blocks in the Udzungwa Mountains (Rovero *et al.* 2012; Rovero *et al.* 2006), the Sanje mangabey has been found only to be present in two isolated forest blocks: Mwanihana forest in the Udzungwa Mountains National Park (UMNP), and the Uzungwa Scarp Nature Reserve forest, limiting the species to <500km^2 habitat (Figure 1.2).

Since its description, the Sanje mangabey has been studied to increase our understanding about its ecology (Ehardt *et al.* 2005; McCabe and Thompson 2013; Fernández *et al.* 2014) and current conservation status (Dinesen *et al.* 2001; Ehardt *et al.* 2005; Rovero *et al.* 2006; Rovero *et al.* 2012; McCabe *et al.* 2019). Since 1994, the Sanje Mangabey Project has focused on the need for researching the species' demographics to help in predicting population trends through long-term monitoring of a habituated group in Mwanihana (Ehardt *et al.* 2005; Fernández, Ehardt, *et al.* 2019; Fernández *et al.* 2014). However, many aspects of the species' biology and ecology remain unknown.

1.1.1. Population Size

Homewood and Rodgers (1981) first estimated the population size from random census walks in only a small area of Mwanihana forest, estimating 1,800 – 3,000 individuals. In 2005, the first recce surveys of

Figure 1.1. An adult Sanje mangabey (*Cercocebus sanjei*) individual in Mwanihana forest, Udzungwa Mountains. Photo credit: C. L. Paddock.

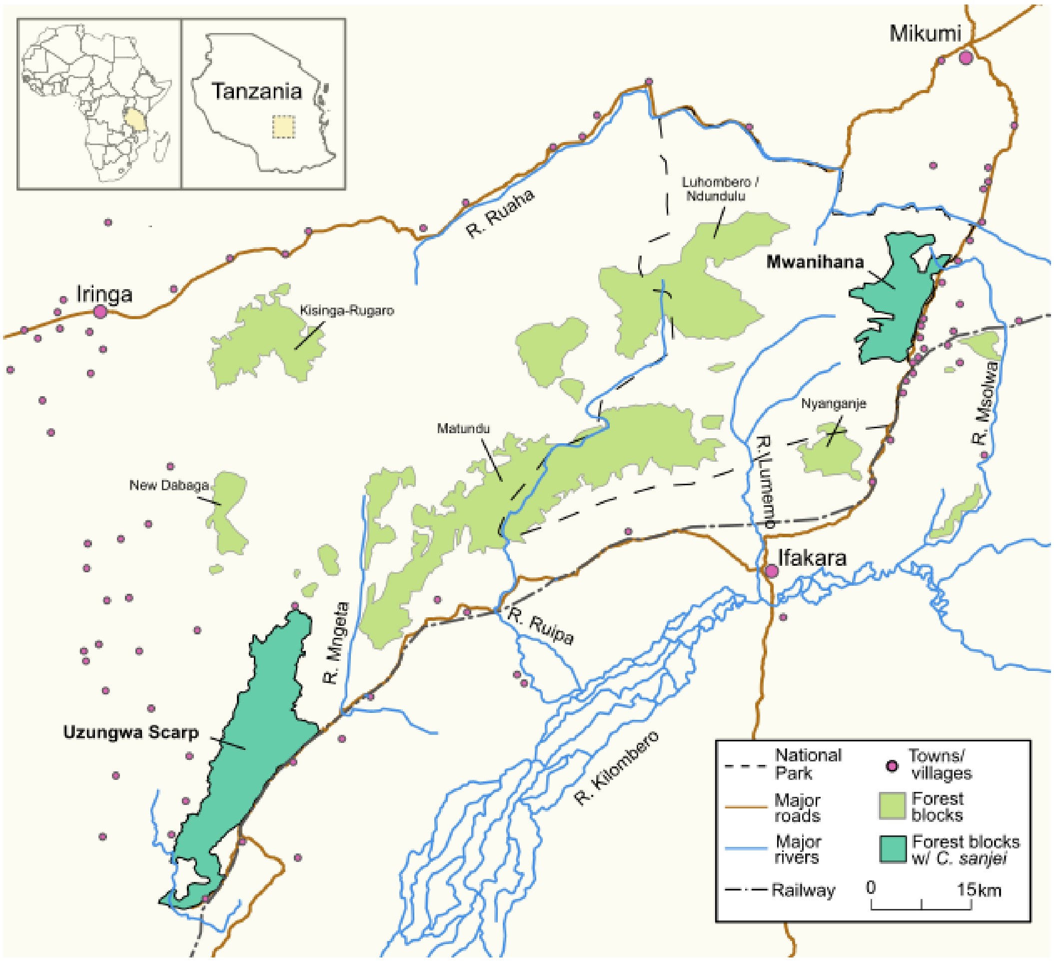

Figure 1.2. A map of the forest blocks within the Udzungwa Mountains, Tanzania, with the two forest blocks in which the Sanje mangabey (*Cercocebus sanjei*) is found: Mwanihana, situated in the north-east within the Udzungwa Mountains National Park boundary, and Uzungwa Scarp, situated in the south-west and the boundary of this forest representing the Uzungwa Scarp Nature Reserve.

the Sanje mangabey estimated <1,500 individuals remaining across the two forests, with predictions of only 40% of the population within the larger Uzungwa Scarp forest (Ehardt *et al.* 2005). From these estimates by Ehardt *et al.* (2005), Rovero *et al.* (2009) adjusted these values to account for a greater average group size, estimated from studies of habituated groups, estimating 2,800 – 3,500 individuals across the two populations, likely the most accurate estimate of population size thus far.

The Sanje mangabey has been included in long-term multi-species primate surveys in the Udzungwa Mountains but often, due to their elusive behaviour and the difficulty to detect groups, were not sighted or thought to be underestimated in the results because of this (Rovero *et al.* 2006; Rovero *et al.* 2012; Rovero and Struhsaker 2007). A study of elevational gradient preferences in Mwanihana forest for the Udzungwa red colobus (*Piliocolobus gordonorum*), Angolan colobus (*Colobus angolensis palliates*), Sykes' monkey (*Cercopithecus mitis*), and the Sanje mangabey, only made 5 sightings of mangabey groups (out of 117 total sightings of primates), too few to be included in the study's statistical analyses (Barelli *et al.* 2014). These sightings were made in submontane and montane regions of Mwanihana forest, with no sightings in lowland regions, which the authors suggested may be attributed to a historic and current avoidance of the greater human disturbance found at lower elevations (e.g., timber and pole cutting; Barelli *et al.* 2014). The accuracy of the population size estimates for the Sanje mangabey is unknown, therefore a systematic survey of the species across its full distribution, with a method appropriate for the species, is required.

1.1.1. Ecology

Sanje mangabey groups are multi-male/multi-female with approximately five adult males within a group of 40-60 individuals (Rovero *et al.* 2009). Females are philopatric in behaviour and solitary males have been observed previously (Ehardt *et al.* 2005)**.** Group home ranges have been observed to cover 4-6km^2 annually and these ranges can overlap with up to three other conspecific groups (Ehardt *et al.* 2005). Conflict between groups is infrequent, however males will call with a territorial 'whoop-gobble' vocalisation to communicate the location of the group to others (Ehardt *et al.* 2005). The daily home range is estimated to be 200ha (Ehardt *et al.* 2005) but is thought to vary depending on food availability. Additionally, the home range has been observed to overlap with three species of diurnal primate; the Udzungwa red colobus, black-and-white colobus, and the Sykes' monkey (Ehardt et al., 2005). The Sanje mangabey is flexible in habitat choice and home range habitats include both primary and secondary forest. The species has been found at a wide range of altitudes from 300-1800m a.s.l. in both mosaic habitats and dense, moist forests (Homewood and Rodgers 1981; Ehardt *et al.* 2005; Dinesen *et al.* 2001; Rovero *et al.* 2009).

Sanje mangabeys are primarily frugivorous and are thought to choose habitat based on the availability of seasonal fruits, but have a flexible omnivorous diet that also incorporates seeds, invertebrates, fungi and flowers, among others (Ehardt and Butynski 2013**.** They are considered semi-terrestrial when foraging, spending approximately 50% of their time on the floor but will spend the remaining time using resources from across all levels of the forest (Ehardt et al., 2005; 2013). Groups are most abundant in mid-altitude, semi-deciduous and mature evergreen forests with a canopy height of 40-50m, although reports have included individuals at lower altitudes (300-500m), in secondary and *Brachystegia* spp. woodland ('miombo'; Ehardt and Butynski 2013).

1.1.2. Evolutionary history

The Sanje mangabey is classified as a *Cercocebus* mangabey; part of the Cercopithecidae family and Papionini tribe, however no museum specimen or holotype is available (Ehardt and Butynski 2013). The taxon is recognised as monotypic by the International Union for Conservation of Nature (IUCN; McCabe *et al.* 2019) and some previous studies (Kingdon 1997; Groves 2001; Groves 2005; Ehardt *et al.* 2008). However, it has also been described as a subspecies of the Tana River mangabey (as *C. galeritus sanjei*; Homewood and Rodgers 1981; Grubb *et al.* 2003; Ehardt *et al.* 2008). Debate continues on the species status of the Sanje mangabey and whether it is monotypic, requiring morphological and genetic confirmation.

Ancestral *Cercocebus* mangabeys are thought to have dispersed across the central latitudes of Africa, speciating when populations became isolated (Devreese and Gilbert 2015). The origin of both the Sanje mangabey and the Kenyan Tana River mangabey (*C. galeritus*) has been previously unknown but both are hypothesised to have been established after isolation in eastern Africa from a large ancestral population distributed across central Africa (Devreese and Gilbert 2015). Devreese and Gilbert (2015) hypothesised that this may be due to a single dispersal event or potentially from separate events to each of the current locations of the Tana River mangabey and the Sanje mangabey. This route would follow that of *Piliocolobus,* with ancestral Tana River mangabey populations dispersing via a more northern route through northern Tanzania or southern Kenya and the Sanje mangabey via a southern dispersal route, towards and across from the Southern Highlands, Tanzania (Devreese and Gilbert 2015; Ting 2008).

It is thought the historical distribution of the Sanje mangabey would have included lowland forests between the two current forest blocks (Homewood and Rodgers 1981). Dinesen *et al.* (2001) reported a third population in the Ndundulu Forest in the northern region of UMNP, however, no further evidence has been found of the Sanje mangabey in this region since. The report is believed to have been a misidentification of the kipunji (*Rungwecebus kipunji*; Ehardt and Butynski 2006), that was later described in 2003 (Jones *et al.* 2005). Currently, it is unknown if or when other populations of Sanje mangabey may have existed throughout the Udzungwa Mountains and if any of the other forest fragments are suitable to support a viable Sanje mangabey population.

1.1.3. Habitat: Udzungwa Mountains, Tanzania

The Udzungwa Mountains is a mountain range in south-central Tanzania (Figure 1.2) that consists of fragmented forest cover. The region is part of the Eastern Afromontane biodiversity hotspot due to the exceptionally high levels of endemism in the region (Mittermeier *et al.* 2011; Table 1.1.). Myers *et al.* (2000) ranked the Eastern Arc Mountains and Coastal Forests of Tanzania and Kenya hotspot as first out of the 25 hotspots for the number of endemic plants and vertebrate species per unit area. A later biodiversity hotspot assessment ranked the Eastern Afromontane region to have the second highest number of endemic higher vertebrate taxa after Madagascar and the Indian Ocean Islands, with 119 endemic species versus 175 (Mittermeier *et al.* 2011).

Table 1.1. Number of species occurring in and endemic to the Eastern Afromontane Biodiversity Hotspot by class (Mittermeier *et al.* 2011).

Class	Occurring	Endemic
Plants	7598	2356
Birds	1325	110
Reptiles	347	93
Freshwater fishes	893	617
Amphibians	244	75
Mammals	510	52

The Udzungwa Mountains have been considered a primate hotspot (Rovero *et al.* 2015; Barelli *et al.* 2015; Cavada *et al.* 2016) and a conservation priority (Ruiz-Lopez *et al.* 2016; Burgess *et al.* 2007). Davenport *et al.* (2014) considered the Udzungwa Mountains the second most important region in Tanzania for conservation as a 'Priority Primate Area'. Twelve known species of primate reside within the mountain forests (Table 1.2), including two endangered and endemic species: the Sanje mangabey and the kipunji (*Rungwecebus kipunji*; Jones *et al.* 2005).

The Udzungwa Mountains play a crucial role in both the local and national economy, serving as a critical water source for local people, agriculture, and to produce energy by two hydroelectric power stations. These installations have been estimated to provide 70% of the country's hydropower, which is equal to 52.6% of the total energy produced in Tanzania (Tanzania Forest Conservation Group 2005). The forests create a microclimate in the region which contributes to an increase in rainfall whilst forest cover reduces instances of flooding and soil erosion in the region (Ehardt *et al.* 2005). With the fertile land and extensive agriculture in the region, the Kilombero valley is an attractive place to live, with the population of the valley growing 3.4%/year (Harrison 2006).

Table 1.2. List of primate species found in the Udzungwa Mountains, Tanzania, and their International Union for Conservation of Nature (IUCN) conservation status (https://www.iucnredlist.org; correct as of March 2021).
EN = Endangered; VU = Vulnerable; NT = Near Threatened; LC = Least Concern.

Common Name	Scientific Name	Status
Sanje mangabey	*Cercocebus sanjei*	EN
Kipunji	*Rungwecebus kipunji*	EN
Sykes' monkey	*Cercopithecus mitis* ssp. *moloneyi*	LC
Vervet monkey	*Cercopithecus aethiops*	LC
Yellow baboon	*Papio cynocephalus*	LC
Udzungwa red colobus	*Piliocolobus gordonorum*	VU
Angolan colobus	*Colobus angolensis palliatus*	VU
Udzungwa galago	*Galagoides zanzibaricus udzungwensis*	NT
Mountain galago	*Galagoides orinus*	VU
Grant's galago	*Galagoides granti*	LC
Greater galago	*Otolemur crassicaudatus*	LC
Small-eared galago	*Otolemur garnetti*	LC

The Udzungwa Mountains are the largest mountain range within the Eastern Arc Mountains, estimated to span an area of 195,321 ha, equal to 48.1% of all forest within the Eastern Arc Mountains (Newmark and McNeally 2018). Forest blocks within the Udzungwa Mountains range in size from only 12km^2 to over 500km^2, although most are below 25km^2 (Newmark 1998), and range in elevation from 270 to 2,600m a.s.l.. Historical forest cover studies have estimated that the Udzungwa Mountains have lost 76.9% forest cover at elevations between 200 to 2,640m (Hall *et al.* 2009). Between the palaeoecological estimate and the year 2000, forest cover was estimated to have declined from 5,861km^2 to 1,354km^2 (Hall *et al.* 2009). The largest rate of forest lost occurred between 1955 and 1975, where 19.7% of the remaining forest cover was lost at a rate of 0.9% loss per year (from 1,745 to 1,402km^2). Between 1975 and 2000, this rate dropped to 0.13% per year (Hall *et al.* 2009).

The UMNP was established in 1992, increasing the legal protection of 1990km^2 of forest in the north-east of the Udzungwa Mountains. Mwanihana is a well-protected forest block within the UMNP, covering 150.59km^2 (7°40' – 7°57'S, 36°46' – 36°56'E; Marshall *et al.* 2010). Since its establishment, Tanzania National Parks (TANAPA) and World Wildlife Fund (WWF) have worked with local villages along the boundary to Mwanihana to promote sustainable living and to improve the livelihoods of local people (WWF Tanzania Programme Office 2007). Mwanihana was previously inhabited with small villages throughout the forest, with high logging pressures through the 1960s to 1980s (Homewood and Rodgers 1981; Ehardt *et al.* 2005). Although the forest has the highest protection status available, evidence of human disturbance remains, including clearing of land for agriculture and poaching. This is seemingly more pronounced in the northern region, furthest from the National Park Headquarters situated in the southern region of the park (Paddock pers. obs.).

The Uzungwa Scarp Nature Reserve is a 314.48km^2 forest block (Marshall *et al.* 2010) in the south-west of the Udzungwa Mountains (7°39' – 7°51'S, 35°51' – 36°02'E), ranging from 290 to 2144m a.s.l.. Low levels of law enforcement and policing of the region has led to high human disturbance, with all large bodied mammals (over 4kg), such as the African elephant (*Loxodonta africana*), buffalo (*Syncerus caffer*) and leopard (*Panthera pardus*), being intensively hunted to extinction in the early 1970s for the bushmeat trade (Rovero and Jones 2012). The loss of these species led to hunters focussing more on smaller mammals, using snares and pitfall trips. The reserve was upgraded to an IUCN Nature Reserve status (Category Ia) from Forest Reserve in 2017 after pressure from conservation groups in the Udzungwa region. This status aims to reduce human use and visitation to preserve the ecosystem and species for scientific purposes and for education. Unfortunately, this change has had little impact on human encroachment in the region and evidence of logging and poaching in the area remains (Oberosler *et al.* 2019). When the region was originally listed as a Forest Reserve, interviews of local people found only 54% admitted to being aware of the regulations in the region, resulting in intense logging in comparison to the more protected forests in the Udzungwa Mountains (WWF Tanzania Programme Office 2007).

1.1.4. Conservation of the Sanje Mangabey

The Sanje mangabey has been listed as Endangered by the IUCN Red List since the first published assessment of the species in 1988 (McCabe *et al.* 2019). The most recent assessment in 2019 considered the greatest threats to the species to be deforestation for timber and charcoal, and from hunting for bushmeat and to remove pests (McCabe *et al.* 2019). The population and the extent and quality of habitat was considered to be decreasing, however a 'robust count' was yet to be conducted to confirm the population trend (McCabe *et al.* 2019). The species is listed under Appendix II of the Convention on International Trade in Endangered Species (CITES), suggesting the species would be threatened with extinction without controlled trade of specimens, and Class B protection by the African Convention on the Conservation of Nature and Natural Resources.

Major threats to the Sanje mangabey are habitat loss and degradation in the region, especially in the Uzungwa Scarp, with wood harvesting for timber and charcoal (McCabe *et al.* 2019). Burning of the forest in the dry season to facilitate hunting, honey collection or open paths, especially in the higher elevations, causes significant damage and forest loss (Ehardt *et al.* 2005). Previous estimates of human population size in the region predicted that the birth and immigration rates were contributing to an annual population increase of 3 to over 10% in different regions of the Udzungwa Mountains (Ehardt *et al.* 2005). Moreover, many people are attracted to the region because of the good quality agricultural land and climate, which poses threats to the forest resources with this continual population increase.

Furthermore, hunting poses a threat; including snares, shooting and hunting with dogs (Rovero *et al.* 2012), however without effective policing on the ground in the Uzungwa Scarp, the incidence of hunting is estimated to be far greater in that forest fragment (Rovero *et al.* 2012; McCabe *et al.* 2019). Previous reports exist of mangabeys being hunted by local people with dogs and nets (Homewood and Rodgers 1981). Although not considered the target for bushmeat trade and consumption, the semi-terrestrial nature of the Sanje mangabey puts it at risk of capture as by-catch in traps set for small-bodied forest mammals, such as duikers (*Cephalophus* spp.), bush pigs (*Potamochoerus larvatus*) and hyraxes (*Hyracoidea* spp.; Nielsen 2006). Rovero *et al.* (2012) did not find increased incidence of capture with increased numbers of snares reported. However, this was discussed as potentially due to only group encounters being measured and numbers of individuals within groups could have been influenced, especially with such large social groups. Additionally, surveys used visual methods unsuitable for the mangabey and therefore unlikely to be an accurate reflection of the true status of the population. The Sanje mangabey has been reported to be sighted nearby human settlements raiding crops (Ehardt and Butynski 2013), however it has also been reported that mangabeys have been killed because they were mistaken for vervet monkeys (*Chlorocebus pygerythus*) or yellow baboons (*Papio cynocephalus*) and as pests to crops (Homewood and Rodgers 1981). Additionally, the Sanje mangabey is subject to predation by African crowned eagles (*Stephanoaetus coronatus*), lions (*Panthera leo*), leopards (*Pathera pardus*) and hyenas (*Crocuta crocuta*), and they can also be bitten by snakes, such as the Gaboon viper (*Bitis gabonica*), in defensive attacks (Ehardt *et al.* 2005; Rovero *et al.* 2009).

The Sanje mangabey may be considered a flagship species for forest conservation in the Udzungwa Mountains. Primates, especially frugivores, are potential ecosystem engineers (Chapman *et al.* 2013) that play a crucial role in the functioning of their habitats for the benefit of both fauna and flora in that region. Previous studies have found that the loss of frugivores in habitats has led to reduced seed dispersal (Markl *et al.* 2012; Bufalo *et al.* 2016), plant fitness (Stevenson *et al.* 2014), plant genetic diversity (Giombini *et al.* 2017), biomass (Peres *et al.* 2016), and plant recruitment and regeneration (Stevenson 2011; Anzures-Dadda *et al.* 2016). The conservation of the Sanje mangabey is not only of importance to protect the species itself but for the ecosystem services that they provide. Their diet consists of mostly fruits (~50%), seeds and nuts (27%) and flowers (2%; Ehardt and Butynski 2013) and their faeces consist mostly of intact seeds with little faecal material (Paddock pers. obs.), suggesting that mangabeys are important in seed dispersal. Their relatively large home range also supports the dispersal of seeds over a long distance which can encourage the regeneration of degraded forests in disturbed and fragmented habitats (Chapman *et al.* 2013).

The Sanje Mangabey Project

Early work from The Sanje Mangabey Project found much of the threat to the species stemmed from conflict between local communities and the enforcement of restrictions that excluded communities from protected areas, creating a disconnect (Fernández, Ehardt, *et al.* 2019). From the establishment of the National Park in 1992 until the ban was lifted in 2011, only local women could enter the protected area to collect firewood and only once a week (Fernández, Ehardt, *et al.* 2019). This led to protests from local communities who wanted free access to the forests' resources, leading to fires being set in the park and high conflict between authorities and communities.

Local communities did not know about the Sanje mangabey and therefore efforts were made by the Sanje Mangabey Project team to focus on conservation efforts engaging the local community and school students in awareness of the species (Fernández, Ehardt, *et al.* 2019). Combining the need for further scientific knowledge of the Sanje mangabey and the engagement of the local community led to the creation of jobs for local people to work within the Sanje Mangabey Project, monitoring the habituated group and collecting life history data. This engagement and opportunity of training and income from the National Park aimed to improve the relationship between local authorities and communities. Further work was done engaging the community by visiting and delivering talks to local school students about the importance of the National Park and the Sanje mangabey (Fernández, Ehardt, *et al.* 2019).

Conservation management plans of species in the Udzungwa Mountains have included recommendations for restoring the connectivity of forest blocks within the Udzungwa Mountains by establishing wildlife corridors (Museo Tridentino di Scienze Naturali 2007; WWF Tanzania Programme Office 2007; Rovero and Jones 2012). Three corridors are recognised within the region including the Mngeta corridor, linking the Udzungwa Scarp Nature Reserve to the northern region of the Udzungwa Mountains, and the Ruipa and Nyanganje corridors that connect the northern region to the Selous Game Reserve and are critical for gene flow for the African elephant (*Loxodonta africana*) populations present (Rovero and Jones 2012). The area is controlled by the central Tanzanian government and has the potential to be fully restored with an increased protection status.

Schemes to promote ecological monitoring by local communities has also been put in place. The Udzungwa Ecological Monitoring Centre (UEMC) was set up in 2006 to manage both monitoring of the National Park, such as the long-term primate monitoring programme, and to establish education programs with the local communities and schools. This has included the training of local people to conduct monitoring studies to survey large mammals along transects, and to measure signs of cutting and human disturbance (Rovero and Barelli 2017). The UEMC has been under management by Tanzania National Parks (TANAPA) since 2017 in an aim to continue the long-term monitoring with the Tropical Ecology Assessment and Monitoring (TEAM) Network programme, training of TANAPA employees, and to promote the community focussed conservation and eco-tourism schemes (Rovero and Barelli 2017). Further conservation programmes are actively present in the Udzungwa Mountains, working towards the protection of the habitats, such as the Southern Tanzania Elephant Program (STEP; Southern Tanzania Elephant Program 2020) and the Udzungwa Forest Project, focussing on the conservation of Magombera forest (Flamingo Land Ltd.; Rovero *et al.* 2009).

A consensus opinion within the conservation community of the Udzungwa Mountains is that the viability of the Sanje mangabey (and other species within the fragmented landscape) would benefit greatly from the improved protection of the Uzungwa Scarp Nature Reserve (Oberosler *et al.* 2020; Rovero *et al.* 2012; Ehardt and Butynski 2013). In June 2019 a workshop was held to focus on the conservation of the 'Mangadrills'; the clade that includes all *Cercocebus* mangabeys, and mandrills and drills (*Mandrillus*; Fernández, Dempsey, *et al.* 2019). The focus of the workshop was to begin the development of the *Mangadrill Conservation Action Plan*. This plan aimed to engage stakeholders by: i) building capacity for conservation and research for range country nationals, ii) promoting education for women and family planning, and iii) promote conservation education and sustainable living in local communities. Additionally, scientific research to increase the understanding of the species was included, prioritising the need for population surveys, and population genetic studies. Furthermore, conservation actions were proposed for each species, with site specific recommendations (Fernández, Dempsey, *et al.* 2019).

1.2. Conservation Genetics

The field of conservation genetics studies the genetic diversity of threatened species and populations, and investigates genetic processes such as genetic drift, adaptation and inbreeding depression (Frankham 2010). This information can be applied in conservation to measure the effective population size and genetic diversity and structure of populations to infer their viability (Shafer *et al.* 2015). The demographic stochasticity of small, isolated populations combined with factors such as inbreeding and a reduction in genetic diversity, adaptive potential and/or an individual's fitness, can result in an extinction vortex (Gilpin and Soulé 1986). As population size decreases the impact of each of these factors increases over time, increasing the risk of extinction.

Effective population size (N_e) is considered one of the most important population parameters in conservation genetics. N_e is the 'ideal population size' that would lose genetic diversity at the same rate as the true census population size (N), with the assumptions that: i) mating is random between individuals, ii) individuals from different generations do not mate, iii) no mutations, iv) an isolated population with no gene flow, and v) all adults are able to mate (Wright 1969). N_e is often lower than N in most wild populations and a low effective population size is particularly harmful in small, isolated populations. Low N_e can contribute to a decrease in population size, again increasing the chance of negative effects from genetic drift and demographic stochasticity (Gilpin and Soulé 1986; Hoban *et al.* 2021).

Random genetic drift can lead to changes in allele frequencies over time, changes within isolated populations leading to non-adaptive genetic differentiation, increased homozygosity within populations and an overall reduction in average heterozygosity across populations, and reduced directional selection as changes by chance become more likely (Frankham 2005). The process of losing genetic diversity and increasing isolation of a population can result in inbreeding in populations with highly related individuals more likely to mate with one another, resulting in a reduction in fitness or inbreeding depression (Frankham *et al.* 2001). Inbreeding depression has been known to reduce reproductive fitness of individuals; affecting processes such as sperm production and quality, fecundity, maternal behaviours, and survival at juvenile and adult stages (Keller and Waller 2002). Inbreeding in populations is measured by Wright's inbreeding coefficient (*F*), which estimates the probability that alleles at a locus are identical by descent (Wright 1969). As diversity is lost and homozygosity within a population increases, the chance of homozygosity in deleterious recessive alleles increases. The reduced diversity in small populations reduces the chance to naturally purge these alleles, reducing the fitness of populations (Caballero *et al.* 2016). Additionally, smaller populations generate new mutations at a slower rate than larger populations and the lower diversity lowers the potential for recombination. In long-lived species, such as primates, long generation times may limit responses to rapid changes in the environment. As environments evolve, so do new strains of disease, parasites and competing species, therefore genetic diversity and flexibility is required to be able to adapt alongside these other evolving factors (Blanchong *et al.* 2016). Individuals may be required to evolve new phenotypes in which adaptations are present to cope with the digestion of a new diet, changing altitude of suitable conditions that may have increased or decreased altitude, and immune responses (Hoelzel *et al.* 2019).

Isolation of populations over long periods of time with little chance of dispersal and gene flow can lead to genetic differentiation. Each subpopulation is then vulnerable to genetic drift and inbreeding if isolated and small in size over prolonged periods of time. The crossing of the diverged populations can potentially result in a reduction in fitness or outbreeding depression (Frankham *et al.* 2011). Examples of outbreeding depression are suggested to be relatively rare in large animals, but has been seen in a few species, mostly where divergence into different species or subspecies was not known prior to crossing (Frankham 2015). It is thought that crosses between populations with fixed chromosomal differences or adaptive genetic differences following long-term isolation are the primary drivers of outbreeding depression (Frankham *et al.* 2011). For example, the Tasmanian devil experienced a population bottleneck prior to its isolation to its distribution in Tasmania losing diversity at both neutral loci and loci associated with immune response (Hendricks *et al.* 2017). The population has declined since the 1990s due to a transmissible cancer, the devil facial tumour disease. A captive population was established in 2006 from populations free of the disease, although the genetic susceptibility is likely to be present, and translocation has been a consideration to supplement wild population sizes. However, evidence has been found of a rapid evolutionary response to the disease at the genomic level in wild populations, therefore the introduction of individuals with susceptibility to the disease may interrupt this response, counteracting natural selection and causing long term consequences (Hohenlohe *et al.* 2019).

For populations that do not show significant differentiation and potential negative results from crossing, conservation efforts can be made to alleviate the pressures on small and isolated populations by introducing measures to promote gene flow within the species. Gene flow between populations can be initiated by the introduction of forest corridors to promote dispersal between isolated subpopulations or by translocations of individuals from different populations. Increasing gene flow aims to reduce the levels of inbreeding by introducing less related individuals to a population and to increase the genetic diversity. The Florida panther (*Puma concolor coryi*) is a success story of genetic rescue with eight female individuals from Texas of a different subspecies, *P. c. stanleyana*, were translocated to a struggling small population of approximately at least 22 individuals of the 'original' Florida panther and four Everglades Florida panthers. From 1995 to 2007, the effective population size increased from 16.4 to 32.1 and increased survival was linked to individuals with an admixed ancestry (Johnson *et al.* 2010). Extinction was estimated to be at a 95% likelihood within two decades in 1995, however translocation combined with the increased protection of habitat from the 1980s led to the tripling of population size and observed behaviours that may be linked with increased fitness (Johnson *et al.* 2010).

1.2.1. Non-invasive Sampling

Using genetics to inform species conservation requires the collection of host DNA samples from species often that are listed as threatened and endangered. Collecting samples from these wild animals can pose both ethical considerations and logistical constraints. The collection of high-quality DNA requires sampling from invasively collected material (e.g. blood and tissue), usually acquired by trapping or sedating of individuals. Sedating and manipulating wild animals requires professionally trained and permitted individuals, usually veterinarians, to be involved in sample collection. Therefore, the collection of non-invasive or minimally invasive samples, such as buccal swabs, hair and faeces, is preferred in conservation genetics and increasingly in conservation genomics (Russello *et al.* 2015).

The risk of injury to the target animal increases and risk of zoonoses is more apparent with physical contact or a closer proximity required for invasive sample collection (Evans *et al.* 2015; Engel and Jones-Engel 2012), whereas non-invasive sampling should result in near to zero risk (Pauli *et al.* 2010). Additionally, the need to observe the species to catch or sedate individuals may be logistically difficult for threatened species that often live at low densities or may exhibit elusive behaviours (Goossens and Bruford 2009). Furthermore, non-invasive sample collection can be logistically easier, especially when studying CITES protected species, as transportation permit processes are generally less constraining (Goossens and Bruford 2009).

Non-invasive sampling of faeces has been widely used to study population genetics and more recently genomics; including phylogeography, phylogenetics, behaviour and population structure (Carroll *et al.* 2018). Although preferable as a source of host DNA, faecal samples have their own limitations in that the DNA that can be extracted usually consists of short and degraded fragments, in low concentrations for host DNA and in high concentration of exogenous DNA (Costa *et al.* 2017). In a study aiming to enrich DNA extracts from the western chimpanzee (*Pan troglodytes verus*), the percentage of endogenous DNA was estimated at an average 1.8%, with the majority of the remaining DNA attributed to the microbiome (Perry *et al.* 2010). The low host DNA concentration also increases the risk of contamination from other species, especially from humans when working with primate samples (Goossens and Bruford 2009). Non-invasive samples have lower quality and concentration host DNA, however, polymerase chain reaction (PCR) can be used to amplify even the smallest traces of DNA, as is the case in studies sampling from museum specimens (e.g., Arandjelovic *et al.* 2009).

1.3. Modelling for Conservation Planning

Conservation management plans are most likely to succeed when information available about a species and its life history and habitat is used to inform actions. With ever changing environmental conditions and rapidly increasing anthropogenic impacts, the responses and consequences of species and habitats must be considered in management planning and in adaptive management responses over time. Computer simulation models can act as an invaluable tool to conservation management planning in assessing current viability for both species' populations and habitats, the impact of conservation threats, and the potential impact of varying conservation strategies. Life history and environmental data can be used in a variety of models to estimate historic, current and future scenarios for different species, and this information can be incorporated into conservation management considerations (reviewed in Hoban *et al.* 2012). Two frequently used models include stochastic population models and ecological niche modelling (ENM). Considering cost implications in conservation management, open-source software is available for both population modelling (e.g. VORTEX; Lacy *et al.* 2018), and ENM (e.g. Maxent; Phillips *et al.* 2004), and therefore these platforms are preferable to costly commercial options.

1.3.1. Ecological Niche Models

Ecological Niche Models use a species current distribution with environmental data at those locations to estimate the ecological parameters associated with the species' presence and requirements. By predicting estimated suitable habitat in current environmental and biophysical conditions, conservation management can be informed of potential survey sites where unrecorded populations may exist. Additionally, potential habitats that would be suitable for reintroduction/introduction of a species if limited for viable habitat in its current distribution.

In addition, models of current presence locations can be extrapolated to estimate historical suitable environments to provide an insight into population demography, such as estimating the time of habitat isolation with changing climate over time or routes of dispersal and gene flow between populations. This can be combined with molecular studies of demographic history for the species to infer evolutionary history and paleodistribution, assuming the physiology of the species has remained constant in that time (Richards *et al.* 2007). From studying the estimated distribution of Hanuman langurs (*Semnopithecus entellus*) during the Last Glacial Maximum (~22,000 YA) by projecting from current day presence, Khanal *et al.* (2018) found corresponding fluctuations in genetic variation and estimated habitat suitability during this time, in response to climatic fluctuations. For conservation, these results highlighted the need for fine detail phylogenetic study of the langur populations to estimate the taxonomy in response to the climatic and physical barriers.

Likewise, current distributions can be extrapolated to future scenarios based on estimations of climate for the locations at that time. This is critical for many species with rapidly changing conditions with climate change and informs conservation actions by identifying priority areas and populations for protection. Hill and Winder (2019) assessed the response of baboon taxa (*Papio* spp.) to estimated future climatic conditions for 2050 and 2070 compared to current day, considering two Representative Concentration Pathways (low: RCP2.6, and high: RCP6.0). They estimated that a warming climate as predicted in those pathways would put three species at significant risk of extinction, with substantial loss of suitable habitat under RCP6.0 conditions, even as species considered to exhibit ecological flexibility. Therefore, this study highlighted the need for protection and conservation action for these species which otherwise would be considered least concern.

1.3.2. Stochastic Population Modelling

Modelling the viability of small populations considers the impact of the processes described in Section 1.2 and predicts the likelihood of the factors combining into an extinction vortex (Gilpin and Soulé 1986). The model provides a quantitative assessment of extinction risks and estimations of the contributing factors and demography. Stochastic population modelling can be used to predict the likelihood of extinction for a species and its resilience to multiple drivers in deterministic and stochastic events, and combinations of both, using both demographic and genetic data (Lacy *et al.* 2018). If species fall below a minimum viable population (MVP) size, often the original cause for decline is no longer the largest threat as extinction dynamics change and other synergistic processes increase extinction risk (Brook *et al.* 2008).

When analysing the population dynamics in mammal populations that declined to extinction, Fagan and Holmes (2006) found population decline rates increased when the time to extinction was shorter, suggesting additional processes, such as inbreeding and low effective population sizes, may have become new primary drivers to extinction. The impact of human activity on populations can be modelled to estimate the impact on population size; such as poaching, habitat loss, habitat isolation and the introduction of disease/invasive species, alongside conservation management plans to alleviate these pressures in the future. By identifying the threats making species the most vulnerable to extinction and management plans that will minimise the impacts most effectively, action plans can be developed to ensure funding is channelled into the plans estimated to be the most effective. Additionally, the severity of extinction threat can be modelled for existing populations to estimate and highlight the response time to prevent extinction required. Stochastic population models have been used previously for a number of non-human primate species to inform conservation management, such as the Raffles' banded langur (*Presbytis femoralis femoralis*; Ang *et al.* 2016), brown howler monkey (*Alouatta guariba*; Angostini *et al.* 2013), Hainan gibbon (*Nomascus hainanus*; Turvey *et al.* 2015) and orangutans (*Pongo* spp.; Utami-Atmoko *et al.* 2019).

1.4. Thesis Aims and Hypotheses

The research conducted and presented in this thesis aims to produce data that will inform conservation management planning for the Sanje mangabey. This includes objectives to fill knowledge gaps about the Sanje mangabey, primarily: i) to conduct the first systematic population survey of the species to estimate the number of individuals remaining, ii) to infer the evolutionary history and systematic and taxonomic status of the Sanje mangabey, iii) to estimate the diversity and structure of the species, and

iv) to conduct a stochastic population modelling for the species and model conservation actions. Below is an outline of each chapter including the background and aims for each:

Chapter Two Estimating the population size of the Sanje mangabey (*Cercocebus sanjei*) using acoustic distance sampling

No previous primate survey in the Udzungwa Mountains had successfully and systematically surveyed the Sanje mangabey and therefore the population size was essentially unknown. This creates difficulties when assessing the conservation status of the species and in monitoring population trends in the species, especially when measuring the impact of anthropogenic or natural events or the success in conservation actions for the species. The Sanje mangabey had been acknowledged to be difficult to detect in dense vegetation during line transect surveys in the region, and therefore a new approach needed to be applied to the species. Furthermore, previous estimates had used estimates for group size and habitat size now considered to be underestimates, therefore inaccurately estimating the population size and likely underestimating also. Chapter Two describes the design and implementation of an acoustic distance sampling method in which the 'whoop-gobble' vocalisation of the Sanje mangabey was used to estimate group density and extrapolate to an estimated population size. This updated and more accurate estimate of group density and population size for each forest will contribute directly to the reassessment of the conservation status of the Sanje mangabey.

Chapter Three An insight into the phylogenetic history of the Sanje mangabey (*Cercocebus sanjei*)

A knowledge gap has existed with respect to the evolutionary history of the Sanje mangabey and its phylogenetic relationship with other *Cercocebus* mangabeys. No previous phylogenetic reconstructions of the Mangadrill clade have included sequences from the Sanje mangabey. Debate remains whether the Sanje mangabey and other central/eastern *Cercocebus* mangabeys are recently isolated and therefore subspecies status. This may have considerable conservation implications for the species considered, therefore Chapter Three conducts a phylogenetic reconstruction of the Mangadrill clade and estimates the time to most recent common ancestor between species. Furthermore, due to other studies in the Udzungwa Mountains estimating isolation of forest blocks and populations on an ancient timescale, the time to most common recent ancestor is estimated between the two Sanje mangabey populations. The results of this study increase our understanding of the Mangadrill taxonomy and will be used to inform conservation management.

Chapter Four Phylogeography and historical niche modelling reveals the evolutionary history of the Endangered Sanje mangabey (*Cercocebus sanjei*)

Investigating the phylogeography of the two Sanje mangabey populations aimed to provide further insight into its evolutionary history and inform conservation planning. Using mitochondrial DNA sequences from individuals sampled across the range of both populations, the study aimed to identify the genetic diversity and structure within and between the populations. Further, the chapter aims to investigate the evolutionary history of the species by estimating its demographic history and whether the populations had experienced recent contraction or expansion in effective population size. Additionally, an ecological niche model was developed for the Sanje mangabey within Tanzania to estimate potential routes of dispersal to and between the current forest fragments. Using the probability of presence and bioclimatic variables most associated with the current distribution of the two populations, this was projected to historic estimates for bioclimatic conditions at three time points: i) Mid-Holocene (~6,000 YA), ii) Last Glacial Maximum (~22,000 YA), and iii) the Last Interglacial Period (~120,000-140,000 YA). The results of this chapter were used to develop conservation actions, determining whether the populations met the criteria to be classified and managed as separate management units or evolutionarily significant units.

Chapter Five Viable or in a Vortex? Stochastic population modelling of the Endangered Sanje mangabey (*Cercocebus sanjei*)

Stochastic population modelling uses information collated from life history data and estimated threats to the population to estimate viability. Further, conservation management scenarios can be modelled to estimate the response of the populations to different approaches. Ecological niche modelling can be used to estimate, from the current presence locations, habitat variables that are estimated to be most correlated with mangabey presence and identify regions outside of the two forest fragments that may be suitable for the species. This study aimed to collate the existing data collected from the habituated Sanje mangabey group and current threats to estimate the viability of the populations if no conservation intervention was made. Using the knowledge developed in the previous chapters, conservation measures deemed appropriate for the species were modelled to estimate the most successful approach for the species. Recommendations for the conservation of the Sanje mangabey were made in this chapter that will be used to inform the conservation action plan being developed for the species in the *Mangadrill Conservation Action Plan.*

CHAPTER TWO

Estimating the population size of the Sanje mangabey (*Cercocebus sanjei*) using acoustic distance sampling

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ABSTRACT

The Sanje mangabey (*Cercocebus sanjei*) is endemic to the Udzungwa Mountains, Tanzania, and is classified as Endangered due to its putatively declining population size, habitat degradation and fragmentation. Previous population size estimates have ranged from 1,350 to 3,500 individuals, with the last direct survey being conducted 15 years before the present study. Previous estimates are now thought to have underestimated the population due to a limited knowledge of group and habitat size, non-systematic approaches and the use of visual methods that are not suitable for surveying the Sanje mangabey with its semi-terrestrial and elusive behaviours. We used an acoustic survey method with observers recording the distinctive 'whoop-gobble' vocalisation produced by mangabeys and point transect distance sampling to model a detection function and estimate abundance. Twenty-eight surveys were conducted throughout the two forests where Sanje mangabeys are found: Mwanihana forest in the Udzungwa Mountains National Park (n=13), and the Uzungwa Scarp Nature Reserve (n=15). Group density was found to be significantly lower in the relatively unprotected Uzungwa Scarp forest (0.15 groups/km^2; 95% CI: 0.08–0.27) compared to the well-protected Mwanihana forest (0.29 groups/km^2; 95% CI: 0.19–0.43; p=0.03). We estimate that there are 1,712 (95% CI: 1,141-2,567) individuals in Mwanihana and 1,455 (95% CI: 783-2,702) in the Uzungwa Scarp, resulting in a total population size of 3,167 (95% CI: 2,181-4,596) individuals. The difference in group density between sites is likely a result of the differing protection status and levels of enforcement between the forests, suggesting that protection of the Uzungwa Scarp should be increased to encourage recovery of the population, and reduce the threat of degradation and hunting. Our results contribute to the reassessment of the species' IUCN Red List status and informing management and conservation action planning.

2.1. INTRODUCTION

Non-human primates are key to the successful functioning of their ecosystems; however, primates are currently facing an extinction crisis with approximately 75% of species declining and 60% threatened with extinction, with the largest threats including habitat loss to agriculture, logging and livestock farming, and hunting (Estrada *et al.* 2017). Research efforts into monitoring wild primate populations have proven crucial in conservation management as recording data on population abundance and distribution can provide insights into the response of a species to changes in habitat and population trends over time (Lwanga *et al.* 2011; Campbell *et al.* 2016; Chapman *et al.* 2017; Jones *et al.* 2019). By establishing an initial baseline and appropriate methodology, these data can be used to assess population trends in subsequent years and develop adaptive management plans that call for the implementation of improved methods to protect species (Lyons *et al.* 2008; Nichols and Williams 2006).

The Sanje mangabey (*Cercocebus sanjei*) is endemic to the Udzungwa Mountains in south-central Tanzania (Ehardt *et al.* 2008). Since its discovery in 1979 by Homewood and Rodgers (1981), it has been studied to elucidate its distribution and population size in order to determine its conservation status and required management (Ehardt *et al.* 2008; Ehardt *et al.* 2005; Rovero *et al.* 2006; Rovero *et al.* 2012), and an inferred declining population size has resulted in an IUCN Red List Endangered status (EN; McCabe *et al.* 2019). The population is divided between two isolated forest blocks: Mwanihana forest in the well-protected Udzungwa Mountains National Park, and the Uzungwa Scarp Nature Reserve forest, which has a lower level of protection and regulations that are not strongly enforced (Ehardt *et al.* 2005). These forests are separated by 100 km of agricultural land and low elevation habitat unsuitable for mangabeys, preventing dispersal of individuals between forests, which could potentially limit the recovery of each population.

The current population size of the Sanje mangabey remains debated and with previous habitat loss and degradation and the current impact of hunting in the forests likely to impact the species, especially in the Uzungwa Scarp (Hegerl *et al.* 2017), current estimates and subsequent monitoring are essential to assess the conservation status and needs of the species. Previous population size estimates range from as little as 1,350 individuals to 3,500 (Dinesen *et al.* 2001; Rovero *et al.* 2009, respectively; Table 2.1) with the last dedicated survey conducted by Ehardt *et al.* (2005) between 1997 and 2002. However, previous studies used methods that were not suitable for the elusive behaviour of the Sanje mangabey, and the group size and habitat area calculations used to extrapolate group density were underestimations, resulting in an underestimated population size.

All previous surveys of the mangabeys have been non-systematic or have used line transect methods to estimate population size. These methods are now recognised to be inefficient for this species as unhabituated groups flee rapidly from humans and are difficult to detect in dense vegetation (Rovero *et* *al.* 2006; Rovero *et al.* 2012; Rovero and Struhsaker 2007). This was supported by line transect observations when individuals were heard calling but were not seen by observers (Rovero *et al.* 2006).

Table 2.1. Previous population size estimates for the Sanje mangabey in the Udzungwa Mountains, Tanzania, and the survey methodology and average group size estimate used to calculate population size.

Previous studies	Survey method	Estimated average group size	Estimated population size
Homewood and Rodgers (1981)	Random census walks in one region	15-20 / 25	1,800-3,000
Dinesen *et al.* (2001)	Recce walks around campsites	15	1,350
Ehardt (2001)	Recce walks along cleared pathways/animal trails	15	<1,300
Ehardt *et al.* (2005)	Refined data from 2001 study using results from the completed 1997-2002 study	10.2 / 13.6	<1,500
Rovero *et al.* (2009)	No survey; updated estimates from Ehardt *et al.* (2005) with larger group size estimate	35	2,800-3,500

The study by Ehardt *et al.* (2005) used a group size of 10.2-13.6 individuals to empirically estimate population size. This value is now thought to be a large underestimate for the Sanje mangabey which has been observed to have groups of up to 70 individuals (G. McCabe pers. obs.). Rovero *et al.* (2009) estimated the population size using the values from Ehardt *et al.* (2005) but adjusted for a larger group size of 35 individuals, which may be more accurate as it is similar to the closely related Tana River mangabey group size (*C. galeritus*: 27 individuals/group; Wieczkowski and Butynski 2013). The total suitable habitat size used by Ehardt *et al.* (2005) is also thought to be an underestimate having used only the closed forest area (Mwanihana: 100 km^2; Uzungwa Scarp: 131 km^2). However, long-term studies of a habituated group in Mwanihana have confirmed that Sanje mangabeys routinely use a variety of habitats; including secondary growth and elephant disturbed shrubland (McCabe *et al.* 2013). Thus, more recent primate surveys have predicted a much larger total forest size with potentially suitable habitat throughout the full extent for Sanje mangabeys (Mwanihana: 150.59 km^2; Uzungwa Scarp: 314.48 km^2; Marshall *et al.* 2010).

Low detection efficiency has been found in other primate species that live in dense rainforests or mountainous regions or are elusive and live at low densities (Lee *et al.* 2015; Marques *et al.* 2013; Plumptre *et al.* 2013). In such species, acoustic methods have been successfully applied, including the black howler (*Alouatta pigra*) and spider monkey (*Ateles geoffroyi*; Estrada *et al.* 2004), indri (*Indri*

indri; Glessner and Britt 2005), and wild cotton-top tamarin (*Saguinus oedipus*; Savage *et al.* 2010). Here, we employ an acoustic distance sampling method to estimate group density for the mangabeys, from which population size can be more accurately extrapolated.

This study aimed to conduct the first systematic survey of the Sanje mangabey population and provide the first inferential estimates of population size for the species. This was the first survey of the Sanje mangabey for 15 years and therefore aimed to establish a more recent and accurate estimate of population size. We used acoustic surveys and a more recent estimate of available habitat size to estimate population size and hypothesised that the lesser protected Uzungwa Scarp would have a lower group density.

2.2. MATERIALS AND METHODS

2.2.1. Study site

Fieldwork was conducted in the Udzungwa Mountains, Tanzania, in the only two forest blocks in which the Sanje mangabey is found: Mwanihana forest (7°40’-7°57’S, 36°46’-36°56’E), situated in the Udzungwa Mountains National Park, and the Uzungwa Scarp Nature Reserve (8°14’-8°32’S, 35°51’-36°02’E; Ehardt *et al.* 2005; Figure 2.1). Data were collected during the dry season between June and November 2017, to minimise the chance of seasonal variation in climatic conditions and species behaviour that may influence detection probability.

2.2.2. Acoustic Survey

A total of 28 survey locations were used to collect vocalisation data for the Sanje mangabey: 13 in Mwanihana and 15 in Uzungwa Scarp (Figure 2.1). Quantum GIS (QGIS; QGIS Development Team 2014) was used to design a systematic grid of points and randomly place this grid on each forest area to select survey locations in regions known to be accessible. Sanje mangabeys have home ranges of 2 km^2 (Ehardt *et al.* 2005); therefore, we aimed to position locations a minimum of 2 km apart to reduce the chance of groups being detected at more than one location.

Sanje mangabey territorial ‘whoop-gobble’ vocalisations have been recorded at distances of up to 1 km (Ehardt *et al.* 2005); therefore, surveys used three listening posts arranged in a 3x1 array, positioned approximately 200 m apart, to allow calls to be detected at more than one post (Figure 2.2a). Distances and bearings between posts were measured using a GPS device (Garmin GPSMAP 54s Handheld Navigator). Posts were not always equally spaced due to the terrain constraints in some locations. When positioning posts, preferential use of ridges was made to reduce the possible obstructions to

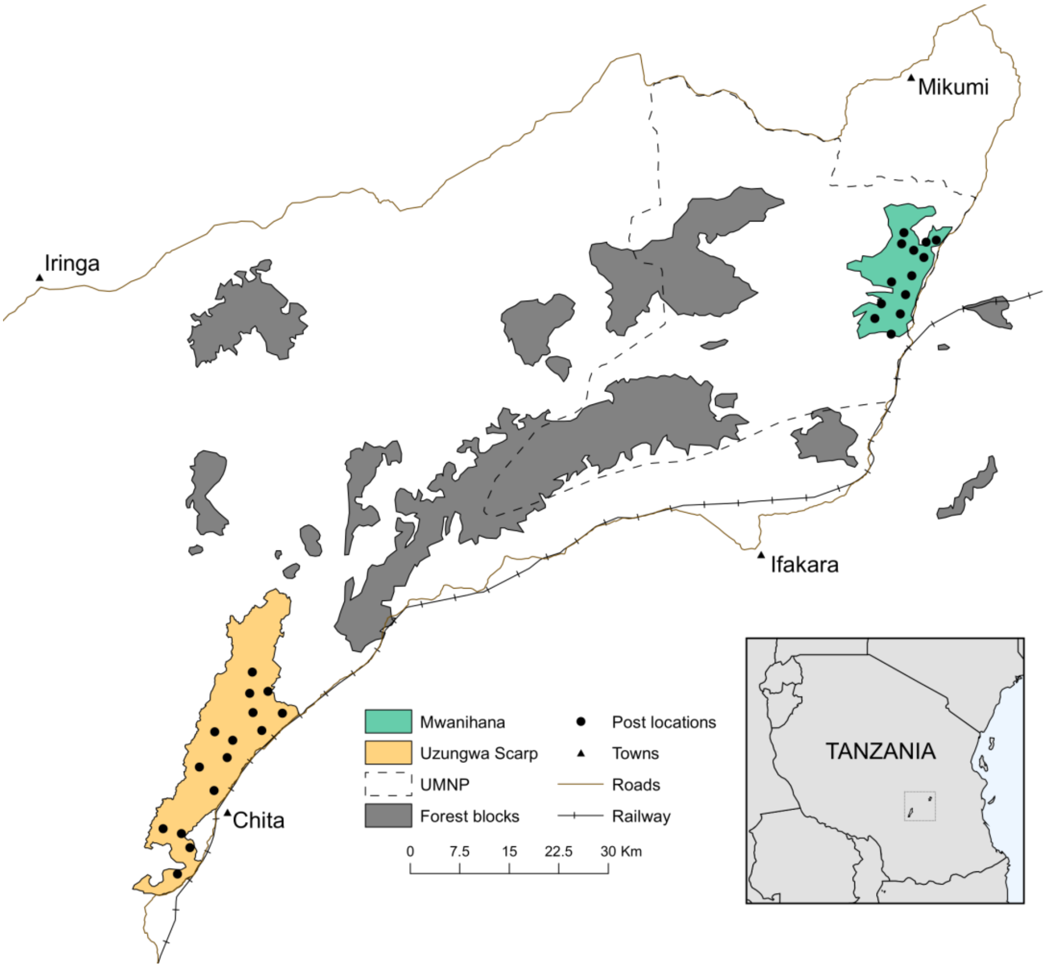

Figure 2.1. Map of the forest blocks in the Udzungwa Mountains and the forest blocks in which Sanje mangabey are found; Mwanihana within the Udzungwa Mountains National Park (UMNP) in the north-east (green), and the Uzungwa Scarp Nature Reserve in the south-west (orange). Listening post locations (circles) are shown at the position of the central listening post.

sound transmission across uneven terrain. This was the most effective use of the total survey effort available and increased the likelihood that the maximum distance individuals could be heard would be similar in all locations. On days of heavy rain, surveys were suspended as the ability to detect calls decreased.

Each survey was conducted once and only early in the morning when the mangabeys are known to call at the highest frequency during the day (approximately 70% of calls before 1200h; Ehardt *et al.* 2005). The surveys started when light levels were safe enough for observers to move through the forest such that survey times were variable. Observations were recorded from the time the observers arrived at the post (mean start time: 0642h ±11.6 minutes) until 0900h; all surveys covered a core time of 0700h to 0900h. The full survey time for each post was used so that the earliest calls (<0700h: 10.2% of calls) were not lost, which would have led to an underestimation of group density.

With each vocalisation detected, observers recorded the time of the start of the call, a bearing from the post and estimated the distance to the origin of the call. Observers would estimate the number of groups heard whilst in the field, attributing individual vocalisations to an assumed group, to support later data analysis. All assistants had been a member of the Sanje Mangabey Project team prior to this study and therefore were well trained and reliable in identifying mangabey calls.

The method followed the assumptions of a point transect survey (Buckland *et al.* 2001). Individuals were detected with certainty at the posts and at the initial location of the call as observers were stationary which ensured groups would not be disturbed and therefore measured at their initial location. The assumption that measurements were exact was not met as distance to calls were estimates by the observers and the variation in terrain and loudness of calls may have affected the perceived distance by observers of each call. Groups could not be located by observers during the survey to validate distances as groups flee quickly if disturbed, making it difficult to locate groups at the original location and risked disturbing other groups. Prior to the study, observers underwent training whilst studying a habituated group to estimate distances and bearings of calls to minimise possible inter-observer differences. The assumption that surveys were positioned at random was violated as listening posts were positioned on nearby ridges and vantage points which may have deviated from randomly assigned points.

A mean average group size for the Sanje mangabey was calculated for each forest from focal follows of five groups (Mwanihana: n=2; Uzungwa Scarp: n=3) found opportunistically when in the field outside survey times, and from known average group sizes of an additional three habituated groups in Mwanihana.

Vocalisations were plotted on a map in QGIS using the bearing and distance estimates recorded during the surveys. Call clusters were used to identify groups in a similar way to previous studies using indri vocalisations to identify distinct groups (*Indri indri*; Glessner and Britt 2005; Pollock 1986). Vocalisations that were within a 300 m distance of another call were assumed to be from the same group. If vocalisations were less than 30 minutes apart and more than 300 m apart, these were assumed to be separate groups (Figure 2.2b). If group definition was unclear (n=13 out of 370 vocalisations) from the plotted vocalisations, notes from the field of assumed number of groups heard were used to attribute the individual calls to a group cluster.

To calculate a detection function and estimated abundance in each forest using distance sampling, the package Distance (Miller *et al.* 2019) was used in R Version 3.5.2 (R Core Team 2018). Survey area was estimated using a fixed radius of 1 km around each post (the furthest distance a mangabey call can be heard; Ehardt et al., 2005) and using QGIS to measure this combined area covered by the three posts. As

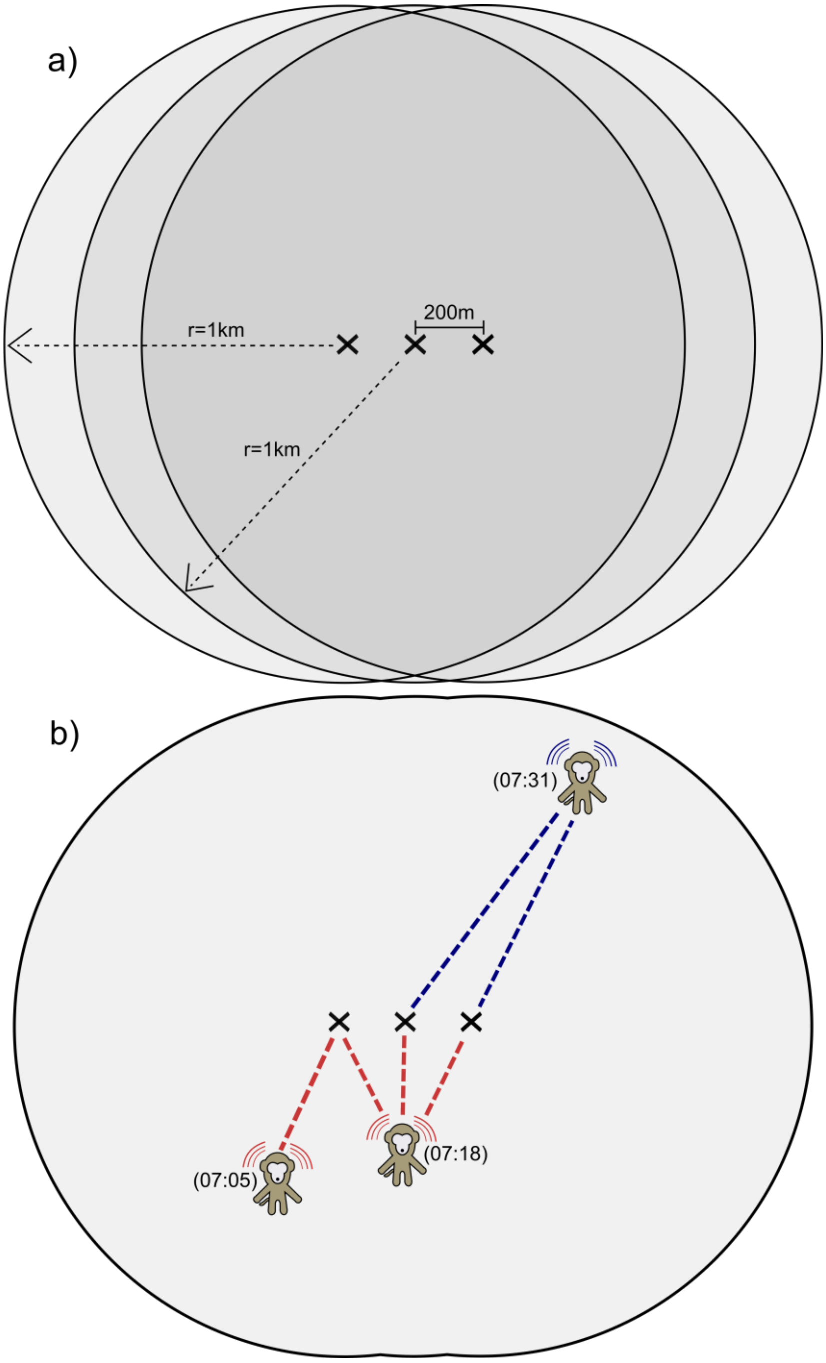

Figure 2.2. Diagrams of the acoustic distance sampling method used in this study: a) The 3x1 array positioning of listening posts with observers (crosses) positioned 200 m apart, with the area of detection for each post (*r* = 1 km; shaded region), and b) An illustration of an example of the call clustering method analysis and attribution of group identification to vocalisations. The time of the call is shown in brackets, dashed lines from posts show the posts that detected the call and the assumed group identification is shown by the colour of lines. The two calls below the posts (red group) are assumed to be the same group as they are close in time and space; less than 30 minutes apart and less than 300 m apart. The call above the posts (blue group) is assumed to be a different group as it is over 300 m away and less than 30 minutes apart from the other calls.

posts were not always equally spaced, this area varied between locations. An average co-ordinate was calculated for each group from all assigned vocalisations and the central point of each survey area was calculated by averaging the co-ordinates of the three listening posts. The distance between this centre point for the survey area and average group position was measured in QGIS to provide a single distance to each group. Group density was calculated for each survey and extrapolated to the total area of suitable habitat from Marshall *et al.* (2010; Mwanihana: 150.59 km^2, Uzungwa Scarp: 314.48 km^2). These estimates were considered the most accurate available as they reflected those found for the predicted suitable habitat area for each forest when using QGIS in this study. The average group size found in this study for each forest was used to inform cluster size in the Distance model to estimate number of individuals.

Observation distances were truncated at 1 km as it is unlikely mangabey calls were accurately detectable past this distance. This removed the detection of 2 groups from a total of 49 detected (4.1% of data) which resembles the removal of the furthest 5% of data suggested by Buckland *et al.* (2001) for point transect surveys. Four detection function models were tested following combinations suggested by Thomas *et al.* (2010) that are considered to perform well and a suitable approach to avoid over fitting with an excess number of models: half-normal key with cosine adjustments, half-normal key with Hermite polynomial adjustments, hazard-rate key with polynomial adjustments and uniform key with cosine adjustments, and the best model was selected using Akaike's information criteria (AIC). The difference between the group density estimates for each forest was measured using a Student's *t*-test, and the difference in group size between forests was measured using a Mann-Whitney U Test. All summary statistics were calculated in R (R Core Team 2018).

2.3. RESULTS

A total of 252 vocalisations were detected in Mwanihana and 118 in Uzungwa Scarp. Using the call clustering method, 32 calling groups were detected in Mwanihana and 17 groups recorded in the Uzungwa Scarp. In Mwanihana, the surveys covered a total area of 100 km^2 and 113 km^2 in Uzungwa Scarp, which is approximately 66.4% and 35.9% of each forest area, respectively, at an average of 7.70 km^2 per survey in Mwanihana (n=13; ±SD 0.40) and 7.51 km^2 per survey in Uzungwa Scarp (n=15; ±SD 0.04). The mean number of individuals per group for Uzungwa Scarp (31.7 ±SD 2.9 individuals; n=3) was lower than Mwanihana (39.2 ±SD 19.4 individuals; n=5), however, the difference was not significant (Table 2.2).

All detection function models fitted well with the data (ΔAIC < 2) and did not differ significantly in abundance estimations. The model using a uniform key with cosine adjustment was selected as the best

Table 2.2. Average group size estimates for the Sanje mangabey in the two forests they occupy: Mwanihana and Uzungwa Scarp in the Udzungwa Mountains, Tanzania, and overall for all groups.

Forest	Number of groups	Group size range	Average group size	± SD
Mwanihana	5	17 - 65	39.2	19.4
Uzungwa Scarp	3	30 - 35	31.7	2.9
TOTAL	**8**	**17 - 65**	**36.4**	**15.3**

fitting detection function model (ΔAIC = 0; goodness of fit: p = 0.46; Figure 2.3). Group density was estimated to be significantly higher in Mwanihana (0.29 groups/km^2; 95% CI: 0.19-0.43) than in Uzungwa Scarp (0.15 groups/km^2; 95% CI: 0.08-0.27; Student's *t*-test: t = 2.25; df = 26; p = 0.03; Figure 2.4). An estimated 43.7 (95% CI: 29.1-65.5) groups and 1,712 (95% CI: 1,141-2,567) individuals were present in Mwanihana. In the Uzungwa Scarp, an estimated 45.9 (95% CI: 24.7-85.2) groups and 1,455 (95% CI: 783-2,702) individuals were found. Therefore, the estimated total number of groups for the Sanje mangabey was 89.6 (95% CI: 60.8-131.9) groups and estimated population size a total of 3,167 (95% CI: 2,181-4,596) individuals (Table 2.3).

2.4. DISCUSSION

The population size estimates in this study are in concordance with previous predictions by Rovero *et al.* (2009), but larger than previous surveys of the Sanje mangabey due to the larger average group size and habitat size sampled in the current study (Dinesen *et al.* 2001; Ehardt *et al.* 2001, 2005; Table 2.3). Ehardt *et al.* (2008) predicted 40% of the population to be residing within Uzungwa Scarp and, here, we again found a very similar proportion, with 46% of the population in Uzungwa Scarp. Ehardt *et al.* (2005) empirically estimated that there were only <1,500 individuals across the two forests, however, when the original density estimates are used in conjunction with the values for habitat size and group size used in this study, now considered a more accurate estimate, total population estimate sizes would have ranged from 4,591–5,536 individuals (Table 2.3). This would suggest a possible decline; however, due to inaccuracies previously discussed of earlier population size estimates, it is not possible to definitively infer a temporal change. Therefore, this study provides the first inferential estimate to allow future surveys to detect and estimate population trends.

Considering population trends from other primates in the same forests, it is likely that there may have been a decline and that the population in the Uzungwa Scarp continues to be at risk of further decline. Populations of primates in Mwanihana have shown to be stable in recent surveys and the active protection measures to be efficient (Rovero *et al.* 2012; Rovero *et al.* 2015; Beaudrot *et al.* 2016), and

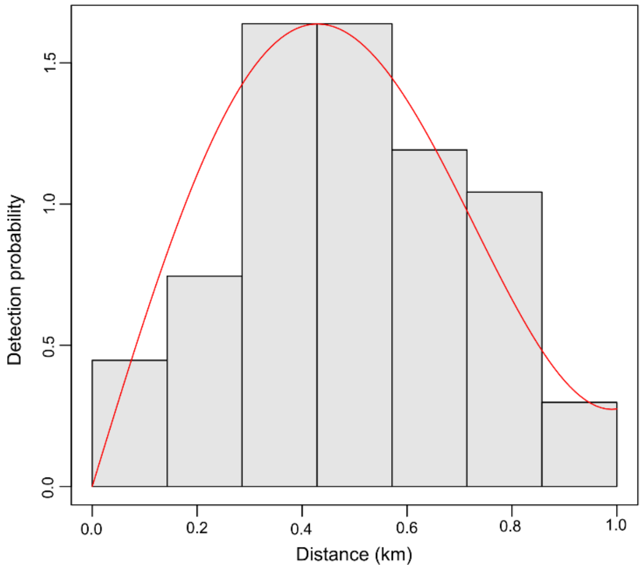

Figure 2.3. The detection function for Sanje mangabey vocalisations detected during surveys of both Mwanihana and Uzungwa Scarp forests in the Udzungwa Mountains, Tanzania. The detection function was estimated using distance sampling in the package Distance (Miller *et al.* 2019), with a uniform model with cosine key estimated to be the best fitting model approach.

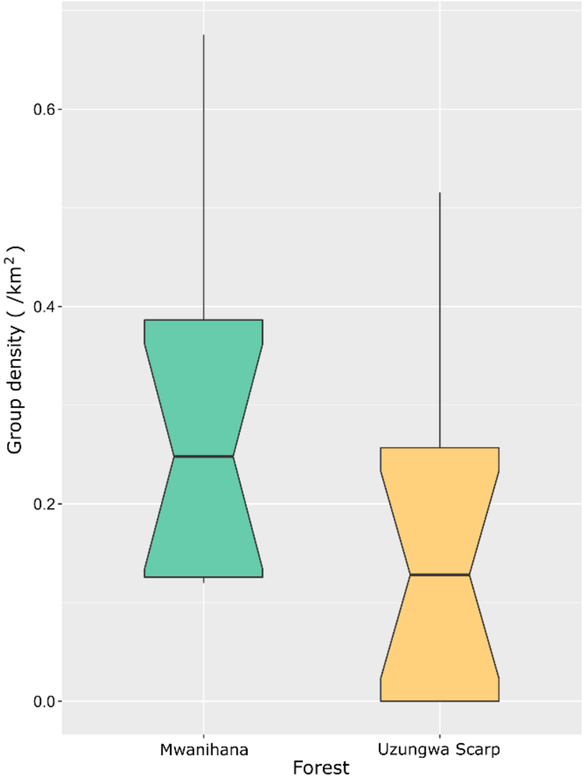

Figure 2.4. A boxplot showing the distribution of group density for the Sanje mangabey in the two forests in which they are found: Mwanihana and the Uzungwa Scarp forests in the Udzungwa Mountains, Tanzania. Group density was significantly higher in the well-protected Mwanihana forest than the lesser protected Uzungwa Scarp (Student's *t*-test: t = 2.25; df = 26; p = 0.03).

although Rovero *et al.* (2012) detected a potential decline in mangabey abundance between surveys in 2004-2005 and 2007-2008 and a survey in 2009, the visual line transect method used was highlighted as inefficient for the mangabey and results to be taken with caution. For the Uzungwa Scarp however, studies report a decline for several primate species (Rovero *et al.* 2012; Rovero *et al.* 2015). In surveys conducted between 2002 and 2012, Rovero *et al.* (2015) found that populations of the arboreal Udzungwa red colobus (*Procolobus gordonorum*) and Angolan colobus (*Colobus angolensis palliatus*) in Mwanihana were stable; however they showed a decline in the Uzungwa Scarp. This was attributed to increased human disturbance in this time period through hunting and pole cutting, both likely to also impact the semi-terrestrial Sanje mangabey.

Group density in Mwanihana was significantly higher than that found in the Uzungwa Scarp, with the lower density found in the forest that presently and historically has had a considerably lower protection status and a clear lack of law enforcement. When using camera traps and occupancy modelling, which is likely an efficient method for the shy, semi- terrestrial mangabey, Hegerl et al. (2017) found Sanje mangabey occupancy in the Uzungwa Scarp was only a quarter of that found in Mwanihana. This difference, as with the difference in group density in this study, suggests that threats to other primates in the Uzungwa Scarp are likely also affecting the Sanje mangabey. Further, findings in this study reflect previous work examining group density for three arboreal primates in the Udzungwa Mountains: the Udzungwa red colobus, Angolan colobus and Sykes' monkey. Across Mwanihana, Uzungwa Scarp, and two other forests, group density of all three species was found to be lowest in the Uzungwa Scarp, which was attributed to the lack of active protection (Araldi *et al.* 2014). Lower densities have often been found for primates living in disturbed habitats compared to those in less disturbed regions due to factors such as reduced biomass, shelter, canopy cover and food availability (Phoonjampa *et al.* 2011). A study by Phoonjampa *et al.* (2011) of pileated gibbons (*Hylobates pileatus*) found group density was significantly associated with habitat disturbance, with higher densities found in forests that had been issued formal protection for longer than those that were more recently elevated.

While both the National Park and Nature Reserve were originally protected by Forest Reserve status, these regulations were weak and often poorly enforced. Mwanihana's protection was upgraded in 1992 when it was included within the Udzungwa Mountains National Park boundary; however, the Uzungwa Scarp was only upgraded to Nature Reserve protection in 2016, which strengthened regulations and management, but did not lead to active patrols or greater law enforcement on the ground. Human disturbance has increased in the Uzungwa Scarp since 2007 (Rovero *et al.* 2010) and the declining encounter rate for the mangabeys has previously been attributed to this escalation in encroachment

Table 2.3. Habitat size, group density and group size estimates used to calculate population size for the Sanje mangabey in the Udzungwa Mountains, Tanzania, for the two forests they occupy: Mwanihana (MW) and the Uzungwa Scarp (US), in the current study compared to previous estimates. Results from Ehardt *et al.* (2005) are reported as the original data presented in the study and as adjusted estimates (new values italicised) where the group densities from the original calculations have been used to calculate population size with the higher group size and habitat size estimates found and used in this study.

Population survey		**Forest**	**Habitat size (km^2)**	**Group density (groups/km^2)**	**Group size**	**Population size**	**Estimated total population size**
This study		MW	150.59	0.29 (95% CI: 0.19 - 0.43)	39.2	1,712 (95% CI: 1,141 - 2,567)	3,167 (95% CI: 2,181 - 4,596)
		US	314.48	0.15 (95% CI: 0.08 - 0.27)	31.7	1,455 (95% CI: 783 - 2,702)	
Rovero *et al.* (2009)		MW	-	-	35	1,750 – 2,100	2,800 – 3,500
		US	-	-		1,050 – 1,400	
Ehardt *et al.* (2005)	Original	MW	131	0.44 - 0.6	10.2 – 13.6	600 - 900	< 1,500
		US	100	0.2		200 - 270	
	Adjusted	MW	150.59	0.44 - 0.6	39.2	2,597 – 3,542	4,591 – 5,536
		US	314.48	0.2	31.7	1,994	

(Rovero *et al.* 2012). A recent long-term study of the impact of protected areas in the Udzungwa Mountains found both species richness and encounter rates for the most commonly encountered medium to large-bodied mammals increased with level of protection status (Jones *et al.* 2019), which further supports the difference in density found in this study for the mangabey.

The acoustic survey method used in this study addressed previous issues from line transect surveys as it did not rely on visual observations and did not disturb the mangabeys that are shy and quick to move away. Therefore, the estimates from this method are likely to be a more accurate representation of the current population size and future surveys of this species should include this approach. Anecdotal observations from the long-term study of the habituated group suggest that it is rare for the groups to not vocalise in the morning (G. McCabe pers. obs.); however, the method in this study could be adapted to bolster estimates by surveying the same location over multiple days to increase detection likelihood. Extrapolating average group density to the full extent of the forest assumed that groups were evenly distributed which may be unlikely given the wide elevation gradient and habitat heterogeneity of both forests. The survey posts were positioned at random and were successful in achieving a mostly full coverage of the forest extent, however, future studies should aim to cover the full extent of each forest and aim to determine whether a difference in group density is found in different habitat types, accounting for possible uneven distribution of groups across forests when estimating population size. Responses to food abundance, quality of forest, habitat structure and proximity to recent human disturbance have been found to influence group density in other studies of primates (Agetsuma *et al.* 2015). The suitability of the habitat and presence of preferred dietary items were not measured in this study but may have had an influence on density within and between forests. The absence of this information means estimates should be considered with caution and assessments of this should be included in future surveys.

No significant difference was found between the average group size for each forest; however, this is likely attributed to the small sample size for each forest and large range of group sizes known from Mwanihana due to two large habituated groups. Future studies would benefit from continuing to estimate group size of all groups encountered to increase the sample size for each forest. In the closely related Tana River mangabey (*Cercocebus galeritus*), a study of the impact of habitat degradation on life history traits found that the subpopulation in a forest of high degradation, due to anthropogenic activities, with lower food abundance had a reduced social group size compared to the subpopulation living with lower levels of habitat degradation (Mbora *et al.* 2009). This was suggested to be attributable to increased parasite prevalence and/or increased competition for food in degraded forest resulting in lower fecundity and increased fitness costs, which may be also applicable in the Sanje mangabey subpopulation in the Uzungwa Scarp with further study.

The Sanje mangabey has shown behavioural and dietary flexibility in its ability to adapt to the use of both primary and secondary forest (Ehardt *et al.* 2005; McCabe *et al.* 2013), which suggests continued and improved protection of the forests to continue the recovery of currently unsuitable degraded habitat to usable secondary forest may encourage an increase in group density. This has been seen in conservation projects aimed at the San Martin titi monkey (*Plecturocebus oenanthe*), for example, where regeneration of forest by increased protection and active reforestation increased group density (Allgas *et al.* 2017). Similarly, increased tree density due to active forest protection led to increased group density for the grey-cheeked mangabey (*Lophocebus albigena*) in the Kibale Forest Reserve, Uganda (Olupot *et al.* 1994).

This study has provided the first inferential estimate of the Sanje mangabey population size which was essential due to previous estimates being considered inaccurate and the last direct survey being conducted over 15 years prior to this study (Ehardt *et al.* 2005). It is key to the survival and protection of species to monitor any changes in the population and the responses to changes in their environment, by natural disaster or anthropogenic disturbances. Populations can be slow to respond to such changes; therefore, long-term and regular monitoring can provide an insight into population trends. Recently, Newmark and McNeally (2018) described the predicted 'sizable' extinction debt due to the fragmentation of forests within the Eastern Arc Mountains, including forests of the Udzungwa Mountains, and the threat to the survival of species within these biodiversity hotspots. Considering this for the Sanje mangabey, we recommend continuing regular population surveys with the acoustic method described here, adapted following recommendations, to regularly monitor the population and to use the results from this study as the baseline population size estimates. The isolation of the two forests preventing migration of individuals and recovery of a population, and the lower group density found in the Uzungwa Scarp, underlines the need for increased protection and active enforcement in this region. Continued active protection of the National Park is required for maintaining the population and potentially aiding an increased group density as highly degraded habitats recover. Active protection of the Uzungwa Scarp needs to be established to prevent the continued impact of hunting and habitat degradation and declining trend in primate populations in the region.

2.5. ACKNOWLEDGEMENTS

Grateful acknowledgements go to Tanzania National Parks (TANAPA), Tanzania Forest Service Agency (TFS), Commission for Science and Technology (COSTECH) and the Tanzanian Wildlife and Research Institute (TAWIRI) for allowing the work to be conducted within the National Park and Nature Reserve. We would like to thank the Sanje Mangabey Project research team, Dr David Fernández, and the staff at the Udzungwa Ecological Monitoring Centre and Udzungwa Forest Project for their assistance with the research. We acknowledge the financial support provided by the UK Natural Environment Research Council (NERC; CASE Studentship NE/N007980/1), Bristol Zoological Society as the NERC CASE industry partner, and Primate Conservation Inc. (#1443) for the fieldwork to be completed.

CHAPTER THREE

An insight into the phylogenetic history of the Sanje mangabey (*Cercocebus sanjei*)

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ABSTRACT

The Sanje mangabey (*Cercocebus sanjei*) is an Endangered primate endemic to the Udzungwa Mountains, Tanzania. The phylogenetic relationship of the Sanje mangabey with other *Cercocebus* species remains unstudied and may have important conservation implications. The status of the Sanje mangabey as a species or a subspecies of *Cercocebus galeritus* has been debated since its description. Here, we reconstruct a phylogeny based on sequences from all recognised *Cercocebus* species, aiming to identify the position of the Sanje mangabey and estimate the divergence time from other species. Phylogenies were reconstructed using nuclear (CD4 and TSPY) and mitochondrial (COII and control region) sequences. The Sanje mangabey grouped with the Central/East African mangabeys; *C. galeritus*, *C. chrysogaster*, and *C. agilis*, however it represents a distinct lineage from these three species, estimated to have diverged from the most recent common ancestor 2.34 million years ago (MYA; 95% HPD interval: 1.73 – 2.95 MYA). The two extant Sanje mangabey subpopulations represent distinct lineages, diverging 0.77 MYA (95% HPD interval: 0.43 – 1.15 MYA). The divergence of these populations is attributed to increasing aridification in eastern Africa beginning ~2.6 MYA, competition and avoidance with other species, and the formation of physical barriers to dispersal, all promoting isolation to montane forest fragments. This study confirms the evolutionary distinctiveness of the Sanje mangabey, therefore conservation management policies should therefore be considered at species level and the subpopulations considered evolutionarily significant units.

3.1. INTRODUCTION

Understanding the taxonomic status of a species can have considerable conservation implications, such as its prioritisation and consideration by international and national organizations, including IUCN Red list classification or local conservation policies (Groves 2014). Published taxonomies can differ between studies and may be considered to be inflated or conflated due to differing approaches to species recognition or inequality of research conducted for different taxa (Lawler 2018), and this confusion can be detrimental in conservation application. For example, as discussed by Stanton et al. (Stanton et al. 2019), a taxonomy of klipspringer (*Oreotragus* sp.) that considers eleven species, as in Groves & Grubb et al. (Groves and Grubb 2011), may require greater conservation resources than a taxonomy considering only one species, as in (Kingdon 1997). However, considering only one species risks undervaluing the species richness present and potentially underestimates loss of genetic diversity. Taxonomic bias can result in finite conservation funds being allocated disproportionally with respect to the evolutionary diversity being conserved (Lawler 2018). Furthermore, cryptic diversity may be present within species that is not signalled phenotypically, but can be discovered when investigating genetic diversity and levels of differentiation, with focus on functional loci and adaptive differentiation (Hoelzel et al. 2019). Therefore, increasing our understanding of phylogenetic relationships and intraspecific genetic diversity is a key step in informing conservation measures.

The Sanje mangabey (*Cercocebus sanjei*; Figure 3.1) was described in the early 1980's (Homewood and Rodgers 1981) and is endemic to the Udzungwa Mountains, Tanzania (Figure 3.2; McCabe *et al.* 2019). It has been listed as Endangered by the IUCN Red List since 1988 with forest fragmentation, habitat degradation and poaching as the primary threats (Oberosler et al. 2019). The Sanje mangabey has been included in the clade of the *'Mangadrills'*, which includes all known *Cercocebus* and *Mandrillus* species (Fernández et al. 2019a). The Mangadrills are distributed throughout west, central and east Africa (Figure 3.3) and comprise nine species; seven *Cercocebus* and two *Mandrillus*. The relative position of the Sanje mangabey and its genetic distinctiveness from the other *Cercocebus* mangabeys remains unknown (Devreese and Gilbert 2015). The Sanje mangabey has not been included in any study of Mangadrill species that investigates morphology, with no monotype or museum specimens available (Wieczkowski and Butynski 2013), or phylogenetic reconstruction, with no genetic sequence data generated previously for the Sanje mangabey (Figure 3.4). Moreover, there has been considerable debate on the status of the Sanje mangabey as a distinct species.

Originally considered a subspecies, as *C. galeritus sanjei* (Homewood and Rodgers 1981), (Kingdon 1997) and (Groves 2001) both argued that the Sanje mangabey was sufficiently morphologically distinct to be upgraded to the species level (Table 3.1). However, (Grubb et al. 2003) argued for the

Figure 3.1. A side profile photograph of the Sanje mangabey showing the white eyelids and backswept crest that resemble *C. galeritus,* and the distinctive beige facial colouring that differs from neighbouring *Cercocebus* populations. Photo credit: C. L. Paddock.

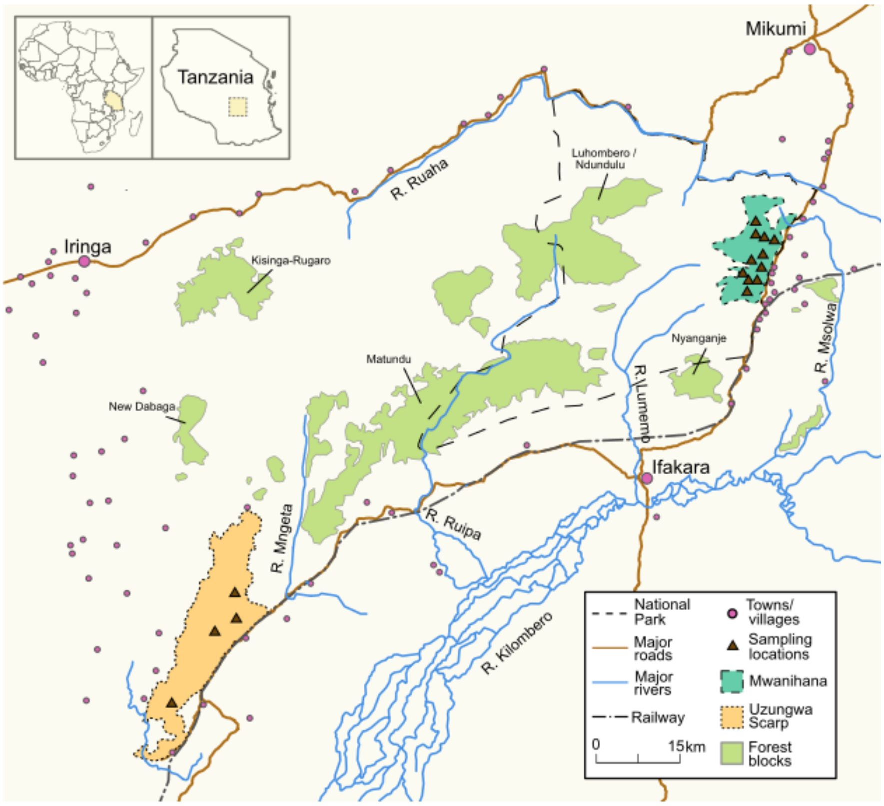


Figure 3.2. Map of the Udzungwa Mountains, Tanzania, with the forests Mwanihana (green; north-east) in the Udzungwa Mountains National Park boundary (dashed line) and Uzungwa Scarp Nature Reserve (orange; south-west) highlighted as the two forest blocks in which the Sanje mangabey (*Cercocebus sanjei*) is found. Sampling locations for faecal samples collected in this study are also marked.

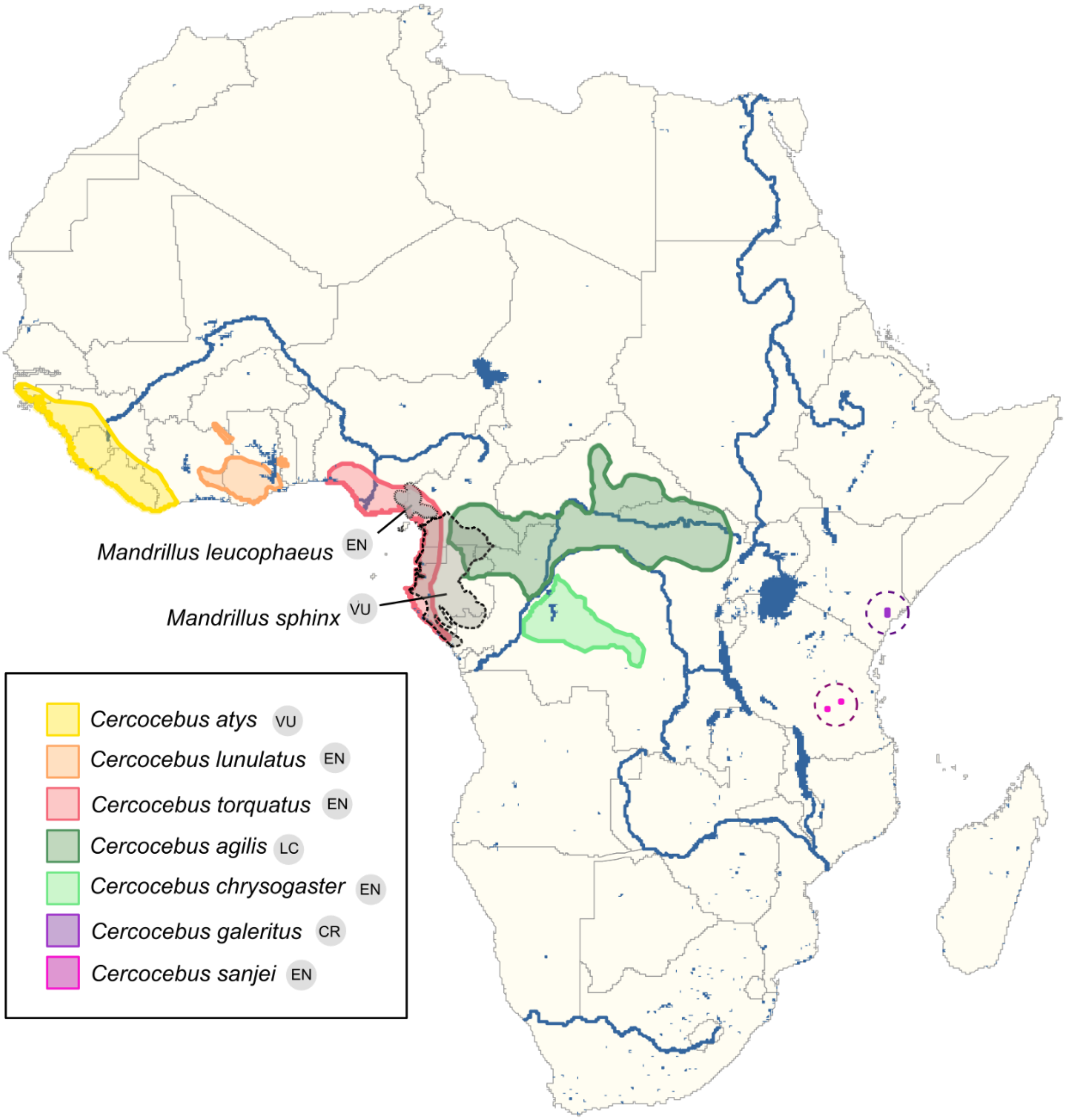


Figure 3.3. Distribution of Mangadrill species throughout Africa, with the distribution polygons downloaded from the IUCN Red List of the seven *Cercocebus* species in colour blocks and the two *Mandrillus* species outlined in greyed areas. The distribution of *C. galeritus* and *C. sanjei* are highlighted with a dashed circle due to the small distribution range of each species. The IUCN conservation status of each species is included in the grey circles: Least Concern (LC), Vulnerable (VU), Endangered (EN) and Critically Endangered (CR). All species have a decreasing tendency of the population size (https://www.iucnredlist.org; downloaded on 18 December 2020).

original subspecies classification based on the observation that the 'whoop-gobble' vocalisations between the Sanje mangabey, agile mangabey (*C. agilis*) and Tana River mangabey (*C. galeritus*) were "*indistinguishable"*. The Sanje mangabey was originally described as most closely resembling the *C. galeritus* subspecies, including *C. agilis* and *C. chrysogaster* as subspecies, with its distinctive whoop-gobble territorial vocalisations, size and shape, sexual dimorphism (males are larger in size and have a longer snout), and shape of the ischial callosities in males and females (Homewood and Rodgers 1981). The face in frontal view, with the direction of hairs around the brow, was described to resemble that of *C. agilis* but the face in lateral view, with a backswept crest, and white eyelids, rather than flesh coloured, more resembling *C. galeritus* (Homewood and Rodgers 1981). The Sanje mangabey pelage was described as the 'intermediate' of *C. galeritus* and *C. agilis*; however the Sanje mangabey differs from *C. galeritus, C. agilis* and *C. chrysogaster* with its face colouration being beige, rather than dark grey/black (Homewood and Rodgers 1981). It was further suggested that the Sanje mangabey may represent an intermediate form between *C. agilis* and *C. galeritus* in biogeographical location, with no evidence of forest mammals colonising the coastal forests in Kenya and Tanzania from a northern route (Homewood and Rodgers 1981).

Table 3.1. Previous nomenclature used to describe the Sanje mangabey as either species level (*Cercocebus sanjei*) or subspecies level (*Cercocebus galeritus sanjei*) in its first description, systematic reviews including the Mangadrills and in previous IUCN Red List reports.

Study		**Nomenclature**
(Homewood and Rodgers 1981)	First description	*Cercocebus galeritus sanjei*
Mittermeier (1986)		*Cercocebus galeritus sanjei*
(Groves 1996)		*Cercocebus sanjei*
(Kingdon 1997)		*Cercocebus sanjei*
(Butynski and Group 2000)	IUCN Red List Assessment	*Cercocebus galeritus sanjei*
(Groves 2001)		*Cercocebus sanjei*
(Grubb et al. 2003)		*Cercocebus galeritus sanjei*
(Groves 2005)		*Cercocebus sanjei*
(Ehardt et al. 2008)	IUCN Red List Assessment	*Cercocebus galeritus sanjei*
(Mittermeier et al. 2013)		*Cercocebus sanjei*
(McCabe et al. 2019)	IUCN Red List Assessment	*Cercocebus sanjei*

Furthermore, the Sanje mangabey is divided into two populations inhabiting two forest fragments, the Mwanihana and the Uzungwa Scarp forests, which are located approximately 100km apart (Figure 3.2). The genetic distinctiveness of these two populations remains unknown. The Sanje mangabey is generally considered forest-adapted (Ehardt et al. 2005) and is likely unable to disperse across the drier habitats surrounding Mwanihana and the Uzungwa Scarp forests. Consequently forest corridors are currently being proposed as a mechanism for dispersal (WWF Tanzania Programme Office 2007). At present it is unknown when the populations and forest blocks throughout the Udzungwa Mountains became separated (Struhsaker et al. 2004).

Efforts have been made to increase our understanding of the Sanje mangabey however, for conservation purposes, further study of population trends, life history, and threats is required (Fernández et al. 2019b; Fernández et al. 2019a; McCabe et al. 2019). The *Mangadrill Conservation Action Plan* currently in development suggests five actions for all species: i) increase stakeholder engagement, ii) reduce existing knowledge gaps, iii) raise the profile of the *Mangadrill* species, iv) enhance protection, and v) habitat restoration (Fernández, Dempsey, et al. 2019). As part of reducing the knowledge gaps, one action highlights the need *'to better clarify the taxonomic and evolutionary relationships between populations'* (Fernández, Dempsey, et al. 2019). So far, studies using morphology and nuclear DNA to estimate the phylogenetic relationship between the Mangadrill species found that Central/West African species (sooty mangabey: *Cercocebus atys*; white-naped mangabey: *Cercocebus lunulatus*; and the collared mangabey: *Cercocebus torquatus*) were broadly distinguished from the species in Central/East Africa (Tana River mangabey: *Cercocebus galeritus*; golden-bellied mangabey: *Cercocebus chrysogaster*; and the agile mangabey: *Cercocebus agilis*; (Disotell et al. 1992; Davenport et al. 2006; Guevara and Steiper 2014; Devreese and Gilbert 2015); Figure 3.4). Exceptions include mitochondrial studies that show paraphyly between *Cercocebus* and *Mandrillus* lineages (Van Der Kuyl et al. 1995; Davenport et al. 2006; Zinner et al. 2011a; Liedigk et al. 2014), or nuclear studies that showed no distinct clades within the Mangadrills (e.g. α 1,3 GT marker: (Disotell et al. 1992; Davenport et al. 2006); TSPY marker: (Disotell et al. 1992); Figure 3.4). However, molecular phylogenetic studies carried out so far have not included sequences from all species (Figure 3.4) and thus the relationship and distinctiveness of each lineage is not known.

Given that eight of the nine species within the *Cercocebus-Mandrillus* clade are under threat of extinction (two considered Vulnerable, five Endangered, and one Critically Endangered), understanding phylogenetic relationships within this clade and of populations within each lineage, supports the identification of cryptic diversity and for effective conservation of the biodiversity present. Therefore, estimating the Sanje mangabey's phylogenetic history and relationship with other members of the Mangadrill clade is a key place to start in informing conservation measures by identifying the distinctiveness of the *Cercocebus* lineages for adequate protection.

Here, the first investigation into the phylogenetic history of the Sanje mangabey is presented. The study had two aims: i) to assess the phylogenetic relationship between *Cercocebus* and *Mandrillus* species using molecular data generated for the Sanje mangabey and published data for other species, and ii) to estimate the time to most recent common ancestor to understand whether the Sanje mangabey represents an evolutionary distinct lineage from other *Cercocebus*.

3.2. MATERIALS AND METHODS

3.2.1. Sample Collection and DNA extraction

Faecal samples were collected between June and November 2017 in the two forests within the Udzungwa Mountains, Tanzania, where the Sanje mangabey is endemic: i) the Mwanihana Forest (7°40'-7°57'S, 36°46'-36°56'E; n = 117 samples), located within the Udzungwa Mountains National Park, and ii) the Uzungwa Scarp Nature Reserve (8°14'-8°32'S, 35°51'-36°02'E; n = 56 samples; Figure 3.2). Twenty-eight locations were searched for mangabeys (corresponding to locations used in an acoustic population survey conducted at the same time (Paddock et al. 2020), with observers climbing to vantage points to listen and locate the distinctive mangabey 'whoop-gobble' vocalisation and then searched for the groups to opportunistically collect faecal samples. When a group was visually detected, the group would be observed from a distance, and observers would follow behind to locate faecal samples.

Samples were only collected if they were 2m apart from the next sample to minimise the chance of resampling individuals. The location and elevation of sampling sites were recorded using Geographic Positioning System (GPS) coordinates (Garmin GPSMAP 54s Handheld Navigator device). Faeces were collected whole and stored until DNA extraction using the 'two-step method' (Roeder et al. 2004): the sample was immersed in 30ml of 97% ethanol in a sterile 25ml universal falcon tube (SARSTEDT AG & Co., Nümbrecht, Germany) for 48h, after which the ethanol was drained and replaced with approximately 10g of Silica Orange (Sigma-Aldrich® Company Ltd., Dorset, UK). The samples were stored in the silica gel at room temperature until DNA extraction.

DNA extraction of the dried samples was carried out in the School of Biosciences, Cardiff University, in a laminar hood. The outermost surface of the faeces was scraped and collected using a scalpel as this material is expected to contain the highest amount of host DNA and the lowest concentration of PCR inhibitors from the diet (Beja-Pereira et al. 2009). DNA was extracted using the QIAamp Fast DNA Stool Mini Kit (Qiagen, UK) and extracts were stored at -20°C until required for analyses. Risk of contamination from exogenous DNA was minimised by ensuring that all DNA extractions were conducted in a laminar-flow hood located in a different laboratory to the PCR setup and with all surfaces sterilised with bleach and ethanol prior to work. When handling samples in collection and processing, gloves, face masks and plastic head caps were worn at all times to prevent contamination with exogenous DNA and to minimise the risk of zoonoses.

3.2.2. Primer Design and DNA Amplification

To estimate the phylogenetic relationship between the Sanje mangabey and other *Cercocebus* mangabeys, two nuclear (autosomal CD4 gene and Y chromosomal testis-specific protein TSPY), and two mitochondrial fragments (cytochrome oxidase subunit II: COII; and control region: CR), were used to reconstruct phylogenies. These fragments were chosen to include freely available sequences produced by previous phylogenetic studies of Papionini (Zinner, Arnold, et al. 2009; Burrell et al. 2009; Olson et al. 2008; Davenport et al. 2006; Tosi et al. 2003; Harris 2000; Harris and Disotell 1998; Disotell et al. 1992). Additionally, the fragments represented three inheritance pathways: mitochondrial maternal inheritance (COII and CR), Y-chromosome inheritance (TSPY) and nuclear autosomal inheritance (CD4). Each pathway can influence the genetic structure of populations differently depending on the life history and dispersal behaviour of a species and thus, phylogenies constructed using each type of inheritance pathway were compared to identify differences in clustering patterns. As no publicly available sequence data existed for the Sanje mangabey at the beginning of the study, primers were designed using conserved regions in alignments of *Cercocebus* sequences obtained from Genbank (Table 3.2). Fragments were targeted using two to three overlapping primers (Table 3.3).

A total fragment size of 401 base pairs (bp) of CD4 (spanning nucleotide positions 127-527 in *C. atys* AF057383) was targeted by amplifying two overlapping fragments of 253 and 351 bp. To design two primer pairs in conserved regions, an alignment of four *Cercocebus* sequences (*C. agilis*: FJ750597; *C. chrysogaster*: AF057382; and *C. atys:* AF057383; AF057384) was constructed. For TSPY, a total fragment size of 621 bp (spanning nucleotide positions 113-733 in *C. galeritus* AY195576) was targeted by overlapping sequences generated by three primer pairs. The primers were designed in conserved regions of nine *Cercocebus* sequences after alignment of sequences from Genbank (*C. agilis*: FJ750633; *C. galeritus*: AY195576; *C. chrysogaster*: AF057409 and AF057410; *C. atys*: AF057411, AF057412, AF057413, and AF057414; and *C. torquatus*: AY195577). A 523 bp fragment was targeted for COII region (spanning nucleotide positions 7,146-7,647 in *C. galeritus* M74004) using overlapping sequences from two primer pairs, which were designed in conserved regions of an alignment of *Cercocebus* sequences from Genbank (*C. agilis*: FJ750650; *C. galeritus*: AY686132 and M74004; *C. atys*: AY686135; *C. torquatus*: FJ713422 and AY686133; and *C. lunulatus*: AY686134). Two primer pairs for mtDNA CR were designed in conserved regions of three *Cercocebus* mitochondrial genomes (*C. torquatus*: NC_023964; and *C. atys*: NC_028592 and KP090062), with the overlapping sequence 515 bp in length (spanning nucleotide positions 15,648-16,218 in *C. atys* NC_028592).

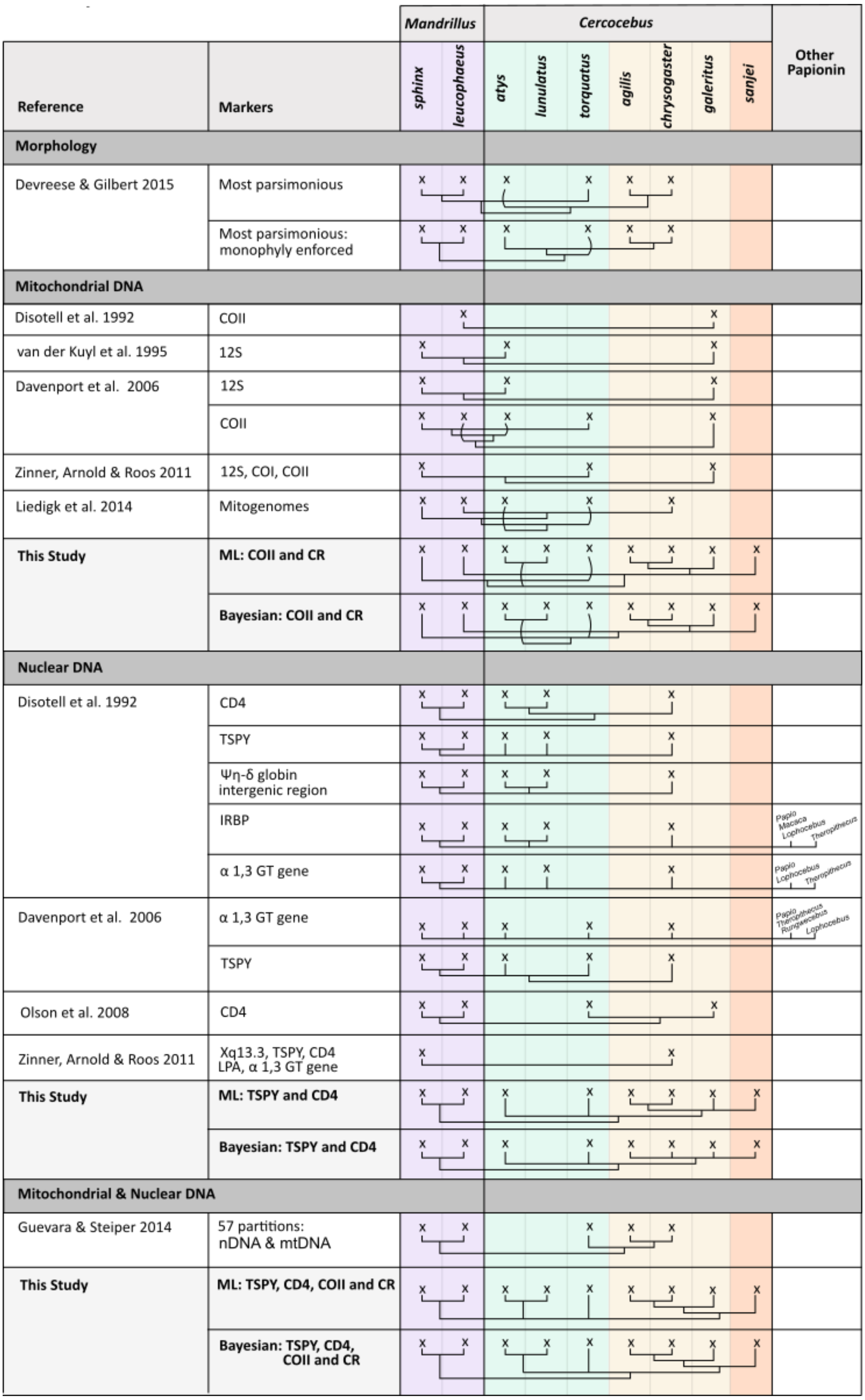


Figure 3.4. A compilation of the most recent craniodental morphological study (Devreese and Gilbert 2015)and the past phylogenetic studies that included at least one *Cercocebus* and one *Mandrillus* species for comparison to the phylogenies from this study.

Table 3.2. Genbank accession numbers for sequences used in this study: one autosomal marker (CD4), one nuclear Y-chromosome marker (TSPY), and two mitochondrial genes; control region (CR) and cytochrome oxidase subunit II (COII).

	CD4	TSPY	COII	CR
Cercocebus sanjei	Generated in this study			
Cercocebus galeritus		AY195576[a]	AY686132[b] M74004	
Cercocebus agilis	FJ750597[a]	FJ560633[a]	FJ750650[b]	
Cercocebus chrysogaster	AF057382[a]	AF057410[a]	NC021943[b]	NC021943[b]
Cercocebus lunulatus			AY686134[b]	
Cercocebus torquatus		AY195577[a]	NC023964[b] AY686133 FJ713422	NC023964[b]
Cercocebus atys	AF057383[a] AF057384	AF057411 AF057412 AF057413[a]	AY686135 KP090062 KT159932 NC028592[b]	KP090062 NC028592[b]
Mandrillus sphinx	AF057386[a] FJ750596	FJ750632[a]	FJ713421[b] FJ750651	KC757403[b] KJ434963
Mandrillus leucophaeus	AF057387[a]	AF057421[a]	M74006[b]	KT696596[b]
Lophocebus aterrimus	AF057390[a] FJ750595	AF057423[a] FJ750631	FJ713415[b]	
Lophocebus albigena	AF057391[a]	AF057425[a]		
Theropithecus gelada	AF057389[a] FJ750594	AF057415[a]	EU293079[b] M74009	NC019802[b]
Rungwecebus kipunji	EU600174[a]	DQ381472[a]	DQ381471[b]	
Papio papio	FJ750590[a] AF057388	FJ750626[a]	EU293078[b]	NC020009[b]
Macaca mulatta	AF057385[a]	AF057416[a] AF425276	M74005[b]	KJ567051[b]

[a]Used in nuclear and full concatenated sequence; [b]Used in mtDNA and full concatenated sequence

Table 3.3. Primer sequences used to amplify fragments of two nuclear loci; CD4 and TSPY, and two mitochondrial genes; control region (CR) and cytochrome oxidase subunit II (COII), in the Sanje mangabey (Cercocebus sanjei).

Region	Primer	Sequence (5' – 3')	Fragment length (bp)	PCR cycling conditions
CR	CRF-1	GCTCCGGGCCCATAACTC	257	95°C for 2 mins, [95°C for 30s, 61.8°C for 60s, 72°C for 2 mins] x 35 cycles, 72°C for 10 mins
	CRR-1	CAAAGACAGGCGCATTCAGG		
	CRF-2	CCRAAACATGCTTACAAGC	343	95°C for 2 mins, [95°C for 30s, 50°C for 60s, 72°C for 2 mins] x 35 cycles, 72°C for 10 mins
	CRR-2	GTTATGGCCCTGAGGTAAG		

COII	COIIF-1	CTATATGCCCTRTTCTCAAC	297	95°C for 15 mins, [94°C for 30s, 50°C for 90s, 72°C for 90s] x 40 cycles, 72°C for 10 mins
	COIIR-1	CTTCTAGGAGTCGAAGGTC		
	COIIF-2	GACYAYGGAGGCCTAATC	303	
	COIIR-2	GTTCYGCRACGATTGG		
TSPY	TSPYF-1	CAGTTGAGAGGTGCTCTTG	264	
	TSPYR-1	CACAGTCCCTTAACAATAGC		
	TSPYF-2	CTGAAGAGCAGAAGCGAG	324	95°C for 15 mins, [94°C for 30s, annealing for 90s, 72°C for 90s] x 20 cycles with annealing starting at 64°C and decreasing by 1°C every 2 cycles, [94°C for 30s, 54°C for 90s, 72°C for 90s] x 20 cycles, 72°C for 10 mins
	TSPYR-2	GGAAGGCCTAAGAGCACC		
	TSPYF-3	CTCAGACACCGGCAGTTC	290	
	TSPYR-3	CATCTTGGTCAGTGATCAGG		
CD4	CD4F-1	CCAAATCCAGCCTGAGCTG	253	
	CD4R-1	CAGCCAAGACAGGGTTTCC		
	CD4F-2	CTGTCAAACTGGCCTCCG	351	
	CD4R-2	GAGTTGGCAGTCACTGTGG		

Each fragment was amplified by Polymerase Chain Reaction (PCR) in a total volume of 10µl, consisting of 5µl QIAGEN Multiplex PCR Master Mix (for CD4, TSPY and mtDNA COII) or 5µl Bioline MyTaq™ (for mtDNA CR), 1µl template DNA and forward and reverse primers at a final concentration of 2µM. All PCR cycling programmes (conditions in) were run in an Applied Biosystems GeneAmp PCR System 9700. The success of each PCR was tested by agarose gel electrophoresis and visualised in a UV transilluminator.

A subsample from each forest were selected for sequencing for TSPY, CD4 and COII based on the amplification success for all primers. A larger number of samples were used (n=64) to generate CR sequences, as this study coincided with a study of phylogeographic structure of the Sanje mangabey subpopulations (Chapter Four). Each unique haplotype sequence found in that study was included in this study. This therefore, will introduce some sampling bias as the diversity of the other markers was investigated in far fewer individuals and may not represent the diversity of the population correctly, however the sequences used still provide the valuable insight into the phylogenetic history required.

Successful amplifications were sent for Eurofins PlateSeq Service (Ebersberg, Germany) and Centre for Molecular Analysis sequencing service (CTM; CIBIO, Portugal) bi-directional sanger sequencing. The chromatogram for all sequences was inspected by eye using Geneious (v4.8.5) to manually trim forward and reverse ends and verify the quality of base calls. Each sequence was submitted to BLAST (Basic Local Alignment Search Tool; https://blast.ncbi.nlm.nih.gov/Blast.cgi) to search against existing sequences in GenBank for the genetically closest sequence and species. Only samples where the top results for percentage identity (>95%) were *Cercocebus* sequences were included in further analyses.

3.2.3. Phylogenetic Reconstruction

To construct phylogenies of the Sanje mangabey within the Papionini tribe, sequences were retrieved from Genbank for *Cercocebus, Mandrillus, Lophocebus, Rungwecebus, Theropithecus* and *Papio* species. *Macaca* was chosen as an outgroup (accession numbers of sequences used in Table 3.2). As previous studies of other species within the Udzungwa Mountains have indicated significant divergence between subpopulations in the Mwanihana and Uzungwa Scarp forest fragments (Ceccarelli et al. 2014; Bowkett et al. 2015; Ruiz-Lopez et al. 2016), sequences from each forest were generated and included as part of separate datasets generated for each forest. Fragments for one Sanje mangabey individual from each subpopulation for CD4, TSPY and COII and all unique haplotypes found for the CR fragment were aligned to sequences for other species. All sequences were trimmed to the length of the smallest sequence to reduce missing data between samples, resulting in an alignment of 400 bp for CD4 (spanning nucleotide positions 126-525 in *C. chrysogaster*: AF057382), 588 bp for TSPY (spanning nucleotide positions 113-700 in *C. galeritus*: AY195576), 480 bp for COII (spanning nucleotide positions 7,168-7,647 in *C. atys*: NC028592) and 369 bp for CR (spanning nucleotide positions 15,689-16,057 in *C. atys*: NC028592).

Maximum Likelihood and Bayesian phylogenies were constructed as each method can show discrepancies in branch support as bootstrap and posterior probability estimates, respectively, and therefore can be used to estimate upper and lower bounds of support (Douady et al. 2003). Three phylogenies were reconstructed with each method using partitioned concatenated sequences: i) concatenating the two mitochondrial fragments (CR/COII; 849 bp), ii) concatenating the two nuclear markers (TSPY/CD4; 988 bp), and iii) all four fragments concatenated (CR/COII/TSPY/CD4; 1,833 bp). To select the most appropriate nucleotide substitution model for each fragment, jModelTest v2.1.10 and the Bayesian Information Criteria (BIC) was used to determine the substitution model to be used for both Maximum Likelihood and Bayesian phylogeny reconstruction (CD4=HKY, CR=HKY+G; COII=HKY+G; TSPY=JC). Maximum Likelihood phylogenies were reconstructed in RAxML v8.2.10 (Stamatakis 2014) with 1,000 bootstrap replicates and generating an extended majority rule tree with a >50% threshold from these replicates. The *Macaca* sequence available in each alignment was assigned as the outgroup. A Bayesian phylogeny with posterior probabilities was generated using MrBayes 3.2.7 (Ronquist et al. 2012) and a Monte Carlo Markov Chain (MCMC) with 1,000,000 generations, sampling every 1,000 and with a 25% burn-in. Convergence of models was evaluated using the potential scale reduction factor (PSRF ~1.0) and effective sample size (ESS >100) for all parameters.

3.2.4. Divergence Time Estimation

To investigate the time to the most recent common ancestor (TMRCA) for Cercocebus species a calibrated phylogeny was created for the concatenated sequence of all fragments (CD4-TSPY-COII-CR;

1,837 bp fragment) using BEAST2 v2.6.0 (Bouckaert et al. 2019). Each fragment was defined as a separate partition with the most appropriate substitution model as estimated for each fragment in the above phylogenies, and individual site and clock models were selected. The divergence tree was constructed using a Calibrated Yule Model, a model appropriate for comparing sequences from different species, and previously published Papionini divergence time estimates as priors to calibrate the model from the phylogenetic history study of the kipunji (Rungwecebus kipunji; Zinner, Arnold, et al. 2009; Table 3.4). A divergence time was included between Cercocebus and Mandrillus, enforcing monophyly, following the strong statistical support found by Devreese and Gilbert (2015) and following the previous study of divergence within Papionins (Zinner, Arnold, et al. 2009).

To calibrate the model in this study, an estimate of the divergence time between Papio and Theropithecus, 3.5 – 4 MYA, from the fossil record was used (Leakey 1993; Delson et al. 2000; Table 3.4). The study by Zinner, Arnold, et al. (2009) estimated the position of kipunji within the Papionini tribe using three of four fragments (CD4, TSPY and COII) also used in this study; therefore these divergence time estimates werealso included in calibrating the model in this study. A normal distribution was used to set the priors across the 95% confidence interval.

The model was run as four independent replicates for 10 million steps, sampling every 1,000 steps with a 10% burn-in, and combined using LogCombiner v2.5.2. Models were inspected in TRACER v1.7.1 (Rambaut et al. 2018) and priors were refined until ESS values were >200. The combined replicates (40,004 trees processed) generated by the model were then summarised in TreeAnnotator v2.5.2 using default parameters and visualised in FigTree v1.4.4 (Rambaut 2018).

Table 3.4. Divergence time estimates and confidence interval used in this study as priors to calibrate a Calibrated Yule Model for the Sanje mangabey (*Cercocebus sanjei*) within the Papionini tribe.

			Divergence estimate (MYA; 95% CI)	**Reference**
Papio	/	*Theropithecus*	3.75 (3.50 – 4.00)	Leakey (1993); Delson et al. (2000)
Macaca	/	*Lophocebus, Theropithecus, Rungwecebus, Papio, Mandrillus & Cercocebus*	9.18 (7.71 – 10.48)	Zinner, Arnold, *et al.* (2009)
Lophocebus	/	*Theropithecus, Rungwecebus & Papio*	4.95 (3.79 – 6.15)	
Cercocebus & Mandrillus	/	*Theropithecus, Rungwecebus, Lophocebus & Papio*	8.47 (6.98 – 10)	
Cercocebus	/	*Mandrillus*	3.73 (2.09 – 5.68)	

3.3. RESULTS

The two nuclear fragments used in this study; autosomal CD4 gene (CD4; 400 bp) and Y chromosomal testis-specific protein (TSPY; 588 bp), showed very low genetic variation between the two Sanje mangabey populations. The CD4 marker had only one segregating site and the alignment showed an average of 99.0% (SD ±0.60) pairwise identity between all species and 97.0% identical sites between sequences. The TSPY sequence was identical in the two Sanje mangabey populations and showed a 98.7% (SD ±0.64) pairwise identity between all species and 94.4% identical sites between sequences. The mitochondrial cytochrome oxidase subunit II (COII; 480 bp) fragment used in this study was identical between Sanje mangabey populations. Comparing between at species level the COII fragment showed a 91.2% (SD ±4.15) mean pairwise identity and 71.9% identical sites between sequences. As expected, the mitochondrial control region (CR; 369 bp) fragment in this study was the most variable sequence studied, with 22 segregating sites between Sanje mangabey populations and a mean 84.7% (SD ±7.76) pairwise identity between all *Cercocebus* species, with 64.2% identical sites between sequences. No evidence of nuclear copies of mitochondrial DNA (NUMTS) was found when assessing the sequences following the recommendations from (Bensasson et al. 2001).

Ten sequences were removed from further analyses following submission to BLAST (Mwanihana: n=3; Uzungwa Scarp: n=7). These sequences showed >95% percentage identity with existing *Cercopithecus mitis* GenBank database sequences. This suggests these samples were collected as a misidentification of Sykes' monkey groups that live sympatrically with the two Sanje mangabey populations.

3.3.1. Phylogenetic Reconstruction

All phylogenies: i) all four fragments; CD4, TSPY, COII and CR, concatenated (1,833 bp; Posterior Probability: PP= 1.00; bootstrap value %: BV= 100; Figure 3.5), ii) COII and CR mitochondrial fragments concatenated (849 bp; PP = 1.00, BV = 98; Figure 3.6a), and iii) CD4 and TSPY nuclear fragments concatenated (988 bp; PP = 1.00, BV = 100; Figure 3.6b), using both a Bayesian and Maximum Likelihood approach for each (six total reconstructions), positioned the Sanje mangabey within a group that included all other *Cercocebus* species (sequences used in each reconstruction are available in Table 3.2).

Phylogenies produced using the concatenation of all four fragments showed strong support for the grouping of *C. sanjei* with *C. agilis*, *C. chrysogaster* and *C. galeritus* (PP = 1.00; BV = 69; Figure 3.5). The two *C. sanjei* subpopulations were, however, reciprocally monophyletic with respect to other Central/East African mangabeys: *C. chrysogaster, C. agilis* and *C. galeritus* (PP = 1.00; BV = 69). Within this group, *C. agilis* and *C. chrysogaster* formed a group distinct from *C. galeritus* (PP = 1.00; BV = 99). The concatenated mitochondrial phylogeny showed strong support for the grouping of *C. sanjei* within a group that included the Central/East African mangabeys and *M. leucophaeus* (PP = 0.99; BV = 64; Figure 3.6a). Furthermore, the *C. sanjei* subpopulations were a distinct lineage from the others within this group. No reciprocal monophyly was evident between *Mandrillus* and *Cercocebus*, with *M. leucophaeus* ingrouped with the four Central/East African species. The concatenated nuclear phylogeny showed support for the grouping of *C. sanjei* with the Central/East African mangabeys (PP = 0.98; BV = 54). The concatenated nuclear phylogeny confirmed the *Cercocebus* group with strong support (PP = 0.98; BV = 54), and in this case showed the expected reciprocal monophyly between *Mandrillus* and *Cercocebus* (PP = 0.98; BV = 54%; Figure 3.6b).

3.3.2. Divergence Time Estimation

Using the concatenated sequence of all four fragments (CD4, TSPY, COII and CR; 1,833 bp) in a Calibrated Yule Model, the Central/East African *Cercocebus* mangabeys (*C. sanjei*, *C. agilis*, *C. chrysogaster* and *C. galeritus*) and the Central/West African mangabeys (*C. lunulatus, C. atys* and *C. torquatus*) were estimated to have diverged 3.35 MYA (95% HPD interval: 2.61 – 3.90; Figure 3.7). The Sanje mangabey lineages were estimated to have diverged from *C. agilis*, *C. chrysogaster* and *C. galeritus* 2.34 MYA (95% HPD interval: 1.73 – 2.95). The Sanje mangabey subpopulations were estimated to have diverged from their most recent common ancestor 0.77 MYA (95% HPD interval: 0.43 – 1.15).

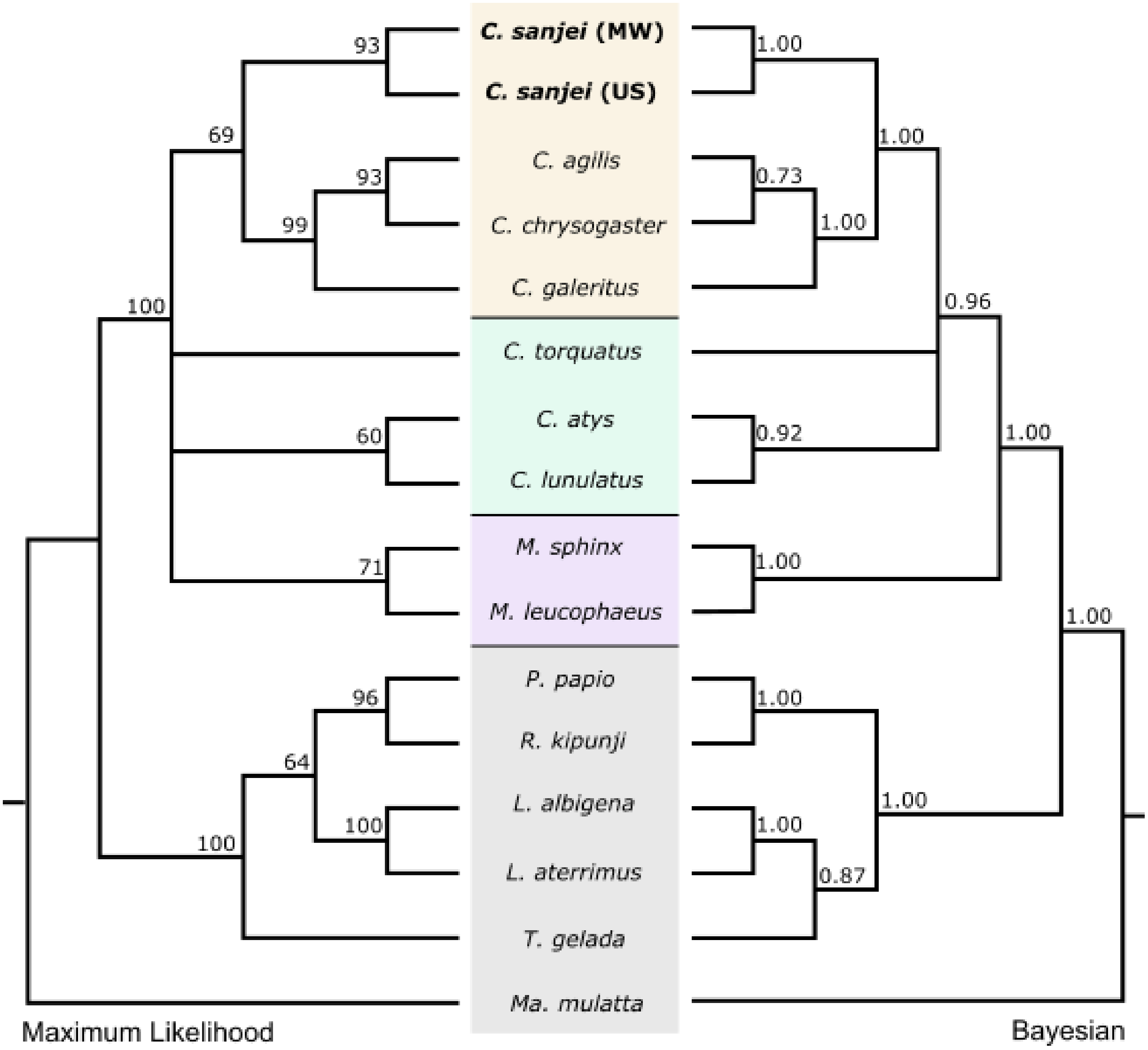
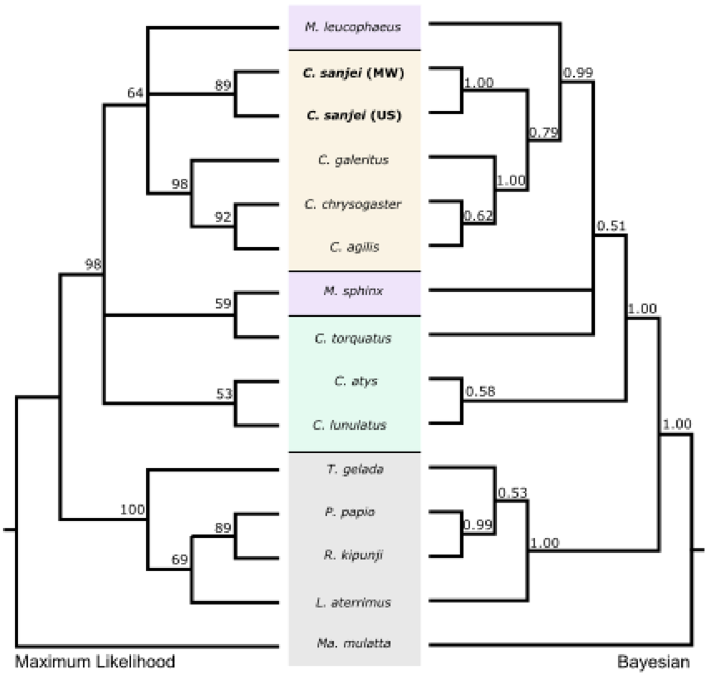


Figure 3.5. Phylogenetic trees for Papionin species using a concatenated sequence (1,833 bp) for four fragments: two nuclear (CD4 and TSPY) and two mitochondrial (COII and CR). Both the Maximum Likelihood phylogeny (left; bootstrap values shown at the nodes) and the Bayesian phylogeny (right; posterior probabilities shown at the nodes) are presented. Sequences used in the phylogeny are available in Table 3.2. *Cercocebus* species in central/eastern Africa, each previously considered subspecies of *Cercocebus galeritus,* are coloured in yellow, the remaining *Cercocebus* species, found in central/west Africa, are coloured green, *Mandrillus* species coloured purple and other Papionin species coloured in grey.

a) Concatenated Mitochondrial DNA



b) Concatenated Nuclear DNA

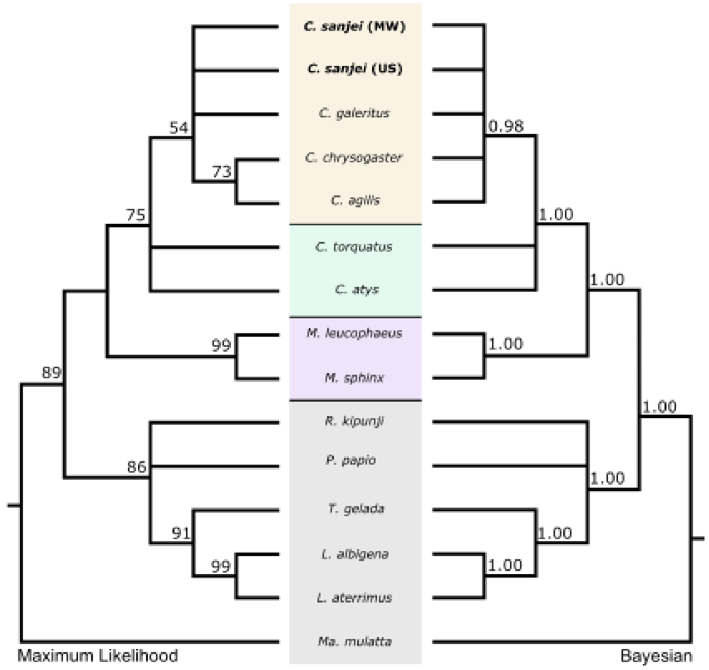


Figure 3.6. Phylogenetic trees for Papionin species using two concatenated sequences: a) mitochondrial cytochrome c oxidase subunit II (COII) and control region concatenated (849 bp) and b) nuclear CD4-TSPY concatenated (988 bp). Both the Maximum Likelihood phylogeny (left of species names; bootstrap values shown at the nodes) and the Bayesian phylogeny (right of the species names; posterior probabilities shown at the nodes) are presented. Sequences used in the phylogeny are available in Table 3.2. *Cercocebus* species in central/eastern Africa, each previously considered subspecies of *Cercocebus galeritus,* are coloured in yellow, the remaining *Cercocebus* species, found in central/west Africa, are coloured green, *Mandrillus* species coloured purple and other Papionin species coloured in grey.

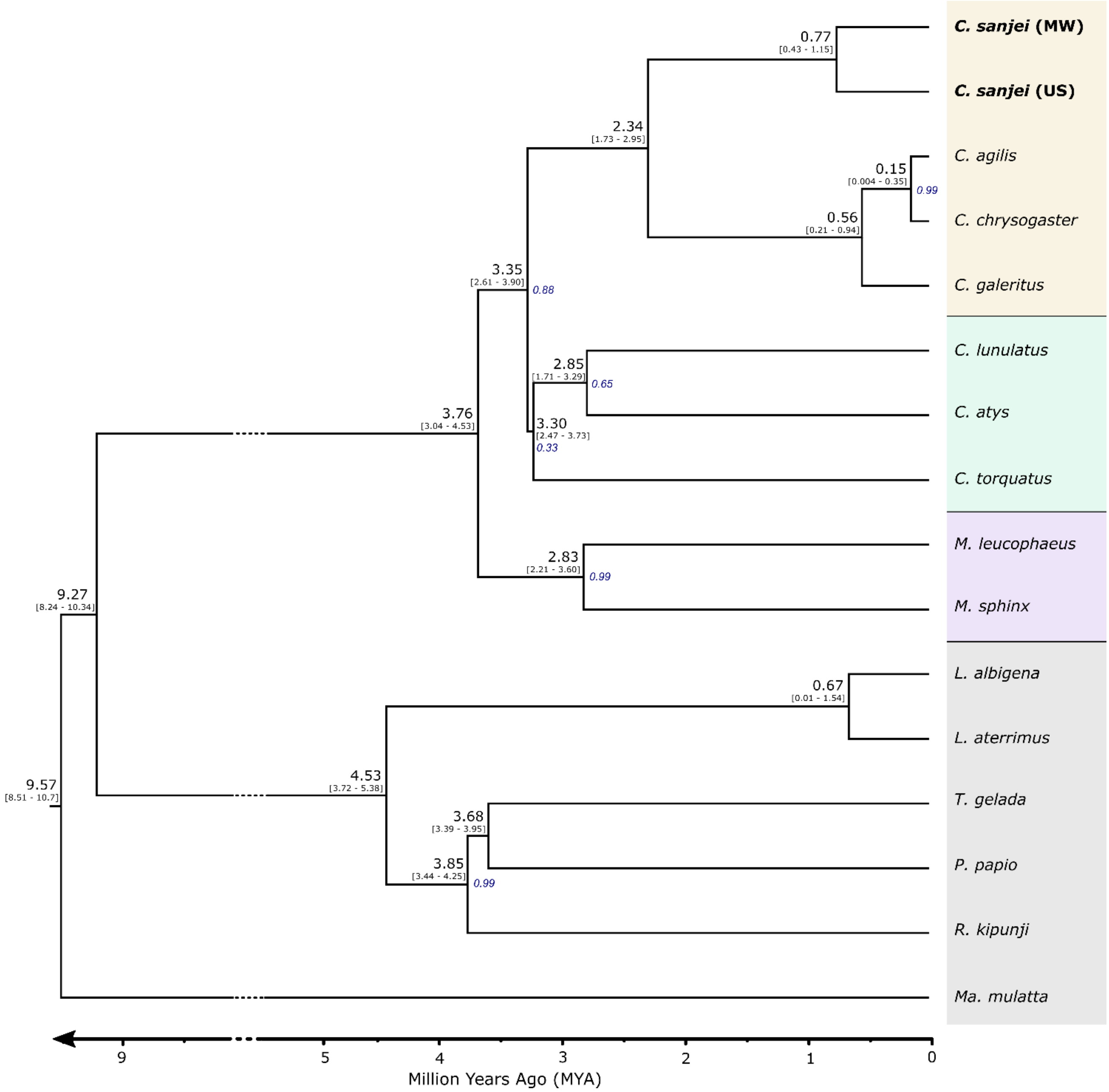


Figure 3.7. Estimation of divergence time in Million Years Ago (MYA) for Papionin species from a concatenated sequence from four genes (CD4, TSPY, and mitochondrial COII and control region; 1,833 bp). Estimated time to the most recent common ancestor (TMRCA) is shown at the nodes with the 95% confidence interval underneath in brackets. Posterior probabilities < 1.00 are shown in italics to the right of the corresponding node. Sequences used in the divergence tree are available in Table 3.2. *Cercocebus* species in central/eastern Africa, each previously considered subspecies of *Cercocebus galeritus,* are coloured in yellow, the remaining *Cercocebus* species, found in central/west Africa, are coloured green, *Mandrillus* species coloured purple and other Papionin species coloured in grey.

3.4. DISCUSSION

This study constitutes the first phylogenetic reconstruction of the Mangadrills (*Mandrillus* and *Cercocebus* spp.) that includes sequences from the Sanje mangabey. In doing so, the relative position of the Sanje mangabey within the *Cercocebus-Mandrillus* clade was clarified and the time to most recent common ancestor with other *Cercocebus* was estimated using four genetic markers (two mitochondrial and two nuclear). The results of the phylogenetic analyses, with all markers concatenated, grouped the Sanje mangabey with the Central/East African mangabeys (*C. galeritus*, *C. chrysogaster* and *C. agilis*), separate from the Central/West African mangabeys (*C. torquatus*, *C. lunulatus* and *C. atys*) with 69% bootstrap confidence and a 1.00 posterior probability. This result recapitulates phylogenetic (Guevara and Steiper 2014) and craniodental studies (Devreese and Gilbert 2015) that also show *C. agilis* and C. *chrysogaster* with a common ancestor, within a clade including *C. atys*, a clade further separate from the *C. torquatus* branch (Figure 3.4).

In the concatenated mitochondrial phylogeny, *Cercocebus* species were paraphyletic with *Mandrillus,* however *Cercocebus* and *Mandrillus* were monophyletic in the concatenated nuclear tree. Incongruence between nuclear and mitochondrial phylogenies can indicate either incomplete lineage sorting or ancient hybridisation and introgression (Petit and Excoffier 2009). Previous studies of mitochondrial phylogeny (Van Der Kuyl et al. 1995; Liedigk et al. 2014), and molecular (Baba et al. 1976; Dene et al. 1976; Hewett-Emmett et al. 1976; Baba et al. 1980; Stanyon et al. 1988), morphological (Fleagle and McGraw 2002; Devreese and Gilbert 2015) and behavioural similarities (Groves 1978) have also indicated a very close relationship between the *Cercocebus* and *Mandrillus* species. Considering previous studies of nuclear and mitochondrial DNA (Perelman et al. 2011; Guevara and Steiper 2014; Davenport et al. 2006; Zinner, Arnold, et al. 2009; Zinner et al. 2011; Liedigk et al. 2014), (Devreese and Gilbert 2015) suggested it may be possible that an ancient introgression event occurred with the overlap of the range of *Cercocebus* and *Mandrillus* in western Africa before dispersal events out from this region.

In this study, the Central/West African mangabeys (*C. torquatus*, *C. atys* and *C. lunulatus*) were estimated to diverge from the Central/East African mangabeys (*C. sanjei*, *C. agilis*, *C. chrysogaster,* and *C. galeritus*) 2.34 MYA (95% CI: 1.73 – 2.95). This time period is coincidental with the time estimated for a *Procercocebus antiquus* fossil (2.0-3.0 MYA; Devreese and Gilbert 2015) discovered in Northern Cape Province, South Africa (Figure 3.8) which showed similarities to extant *Cercocebus* mangabeys with the most craniodental morphological similarities to *C. torquatus* (Gilbert 2007). Devreese and Gilbert (2015) hypothesised that *Cercocebus* species originated in Central/West Africa, with all three suggested scenarios showing an eastern dispersal from either of these distributions towards the current central/eastern mangabeys (Devreese and Gilbert 2015). Devreese and Gilbert (2015) noted that it remains unclear as to whether the *P. antiquus* fossil is representative of an early dispersal route from western equatorial Africa south, or if *Cercocebus* originated in the south and migrated north to diversify into the current *Cercocebus* distributions across Africa. The discovery of more fossils and studies using a greater set of genome wide nuclear loci to test for consistent monophyly in nuclear phylogenies is required to confirm this conclusion.

The group including Central/East African species distinguished in this study included all the sequences from mangabeys previously considered subspecies of *C. galeritus* (*C. sanjei*, *C. galeritus*, *C. agilis* and *C. chrysogaster*). Our nuclear/mitochondrial concatenated phylogeny revealed that the Central/East African species share a common ancestor, the Central/West African *C. atys* and *C. lunulatus* share a common ancestor, and *C. torquatus* was a distinct lineage from these groups. The results of this study support molecular, morphological and behavioural evidence suggesting a close phylogenetic relationship among Sanje mangabey, *C. galeritus*, *C. agilis* and *C. chrysogaster* (Homewood and Rodgers 1981; Kingdon 1997; Groves 2001; Grubb et al. 2003; Devreese and Gilbert 2015). Interestingly, the TMRCA between the Sanje mangabey and *C. galeritus*, *C. agilis* and *C. chrysogaster* was estimated at 2.34 MYA (95% CI: 1.73 – 2.95), which is considerably older than the estimated TMRCA of 0.56 MYA (95% CI: 0.21 – 0.94) for these three *Cercocebus* species. These results suggest that the Sanje Mangabey is an evolutionary distinct lineage within the *Cercocebus* clade.

3.4.1. Dispersal and Divergence in East Africa

Interestingly, the time scale of approximately 2.34 MY (95% CI: 1.73 – 2.95) divergence time of the Sanje mangabey from other *Cercocebus* mangabeys is very similar to that observed in the mitochondrial divergence found for the sympatric Angolan colobus (*Colobus angolensis palliatus*) population in the Udzungwa Mountains from the Guereza colobus (*Colobus guereza*; shared distribution with *C. agilis,* found across most of central equatorial Africa) with an estimated TMRCA at 2.1 MYA (Ting 2008). As a sympatric species, it is likely these two species experienced similar patterns of dispersal leading up to and since this time estimate. This divergence estimate of approximately 2 MY could potentially coincide with a changing climate in eastern Africa (Bobe and Behrensmeyer 2004; deMenocal 2004), the formation of physical barriers to dispersal (such as rivers and rifting activity), and competition and/or avoidance with other species occupying the same regions during this time.

Historical Climate Change

The climate in East Africa throughout the Miocene to mid-Pliocene (3 – 8 MYA) consisted of much warmer and wetter conditions, resulting in an abundance of forest throughout the region (Butynski and Jong 2020). The following Pliocene-Pleistocene eras consisted of drier periods coinciding with glacial cycles at 3.2, 3.0, 2.8, 1.7 and 1.0 MYA, with a gradual shift of vegetation from closed forest to grassland

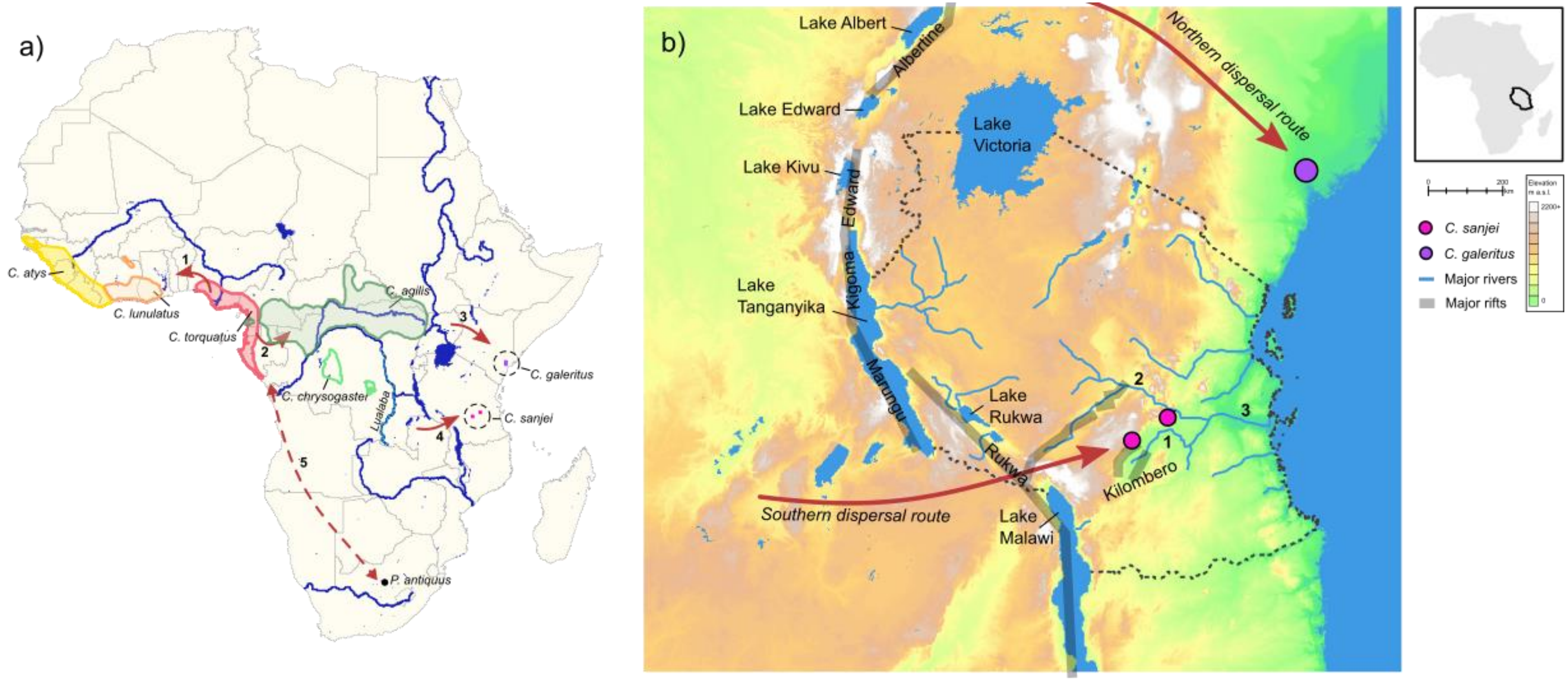


Figure 3.8. Potential dispersal routes for the Sanje mangabey (*Cercocebus sanjei*) to eastern Africa and potential barriers to dispersal: a) the distribution of *Cercocebus* species across Africa with the arrows representing the dispersal from *C. torquatus* west (1) and east (2), creating the eastern and western clades found in this study, and the potential two dispersal events for *C. galeritus* (3) and *C. sanjei* (4). The distribution of *C. galeritus* and *C. sanjei* are highlighted by a dashed circle outline and proposed dispersal route either north from the fossil location *P. antiquus* to *C. torquatus* or south from *C. torquatus* to *P. antiquus* (5); and b) an elevation map of Tanzania and the surrounding regions, including the locations of the major water bodies, rivers and rifts in Tanzania. Three major rivers surround the Sanje mangabey distribution: Kilombero (1), Ruaha (2), and Rufiji (3). Directionality of dispersal is based on the scenario in the craniodental study by Devreese and Gilbert (2015) that is deemed most representative of the results in this study.

3.6 - 2.4 MYA and a significant increase in dry species 2.8 - 2.4MYA (deMenocal 2004). Additionally, from 2.5 MYA onwards, there was an estimated increase in mammal species adapted to grassland, with the appearance of *Homo erectus* (a *Homo* species adapted to dry environments) linked to this grassland expansion after 2 MYA (Bobe and Behrensmeyer 2004). The expansion and contraction of forests and the reduced suitable closed forest habitat available after 2.4 MYA may have influenced dispersal routes between forest fragments, leading to isolation and subsequent speciation. Endemism in the Eastern Arc Mountains has been linked with the stability of ecosystem refuges during periods of climatic change (Fjeldsa and Lovett 1997; Marchant et al. 2007; Mumbi et al. 2008) suggesting ancestral populations of Sanje mangabey may have become isolated in the forests of the Eastern Arc Mountains during these climatic cycles in stable populations.

Physical Barriers to Dispersal

The Albertine Rift may have influenced the evolutionary history of the Central/East African mangabeys. Significant tectonic activity is estimated to have occurred 2 – 3 MYA, with the Gregory Rift (northern Kenya) and the Albertine Rift (western Tanzania) both having major uplift events during this time (Partridge et al. 1995; Figure 3.8b). It is also estimated that in the last 3 MY there has been active rifting across the Rukwa, Albertine and Kigoma basins and rift propagation from Lake Malawi and Lake Tanganyika in a southerly and easterly direction (Macgregor 2015). A palaeolake once extended across the western side of the rift, between the current Lake Albert and Lake Edward 2.5 – 7 MYA and the uplift in this region occurring in the last 2 MY, forming the 'rift shoulder' 2.3-2.6 MYA (Macgregor 2015; Figure 3.8b) and the Rwenzori Mountains 2 MYA (Kaufmann et al. 2016).

The phylogenies of the Central/East African red colobus (*Piliocolobus* sp.) species have been found to be divided into two clades, one including the Kenyan Tana River colobus (*P. rufomitratus*; sympatric with *C. galeritus*) with more central African colobines, and one grouping the Udzungwa (*P. gordonorum*: sympatric to the Sanje mangabey) and Zanzibar red colobus (*P. kirkii*; Ting 2008). A study of the cranial morphology of red colobus species suggested the distinct morphology of *P. rufromitratus*, more closely resembling ancestral central species than the Udzungwa and Zanzibar colobines, suggests two dispersal events into East Africa (Cardini and Elton 2009). This pattern resembles that of the Central/East African *Cercocebus* mangabeys found in this study, with the Sanje mangabey diverging 2.34 MYA (95% CI: 1.73 – 2.95) from *C. galeritus, C. agilis* and *C. chrysogaster*, and these three species having a much more recent TMRCA of 0.51 MYA. They suggest the ancestral Tana River red colobus dispersed north of the Rift Valley and the Lualaba river (Figure 3.8a), through montane refugia across to the Tana forests (Cardini and Elton 2009). The Udzungwa and Zanzibar red colobines dispersed along a southern route from Lake Tanganyika across the Southern Highlands to the Udzungwas and the forests along the Ruaha-Rufiji River, as described for *Cercopithecus* species (Butynski and Jong 2020). Previously, Devreese and Gilbert (Devreese and Gilbert 2015) suggested that *C. galeritus* and *C. sanjei* became relicts *'fairly recently'* however, this study appears to agree with their alternative hypothesis that each species arrived at their current distribution through separate dispersal events following that of *Piliocolobus* (Ting 2008; Devreese and Gilbert 2015). This suggests the ancestral Sanje mangabey may have followed the southern dispersal route away from the other Central/East African mangabeys.

Physical barriers within Tanzania may also have favoured dispersal across the Southern Highlands (Figure 3.8b). A study of baboon (*Papio* sp.) mitochondrial phylogeography found the East African lineages were divided into a clear northern and southern clade, with the Ugalla-Malagarasi and Ruaha-Rufiji rivers likely serving as geographic barriers (Zinner et al. 2015; Figure 3.8b). The study further highlighted that these rivers potentially also act as a barrier between *P. anubis* (northern) and *P. cynocephalus* (southern; Kano 1971), as a southern limit for colobines and the Zanzibar dwarf galago in coastal eastern Africa, and as the northern limit for the Mozambique dwarf galago (Butynski et al. 2013; Figure 3.8b). With the divergence of the Sanje mangabey from other *Cercocebus* species and the proximity of its current distribution to the Ruaha-Rufiji rivers, this suggests these rivers may have acted as a dispersal barrier for the Sanje mangabey as well. Furthermore in baboons, the dispersal event from southern Africa to eastern Africa and divergence into the northern and southern lineages was estimated to have occurred at around ~2.1 MYA (Zinner, Groeneveld, et al. 2009), coinciding with the divergence time of the Sanje mangabey from other *Cercocebus* mangabeys. This was thought to be due to the expansion of savannah and created a subsequent radiation of savannah adapted species such as antelope (Bovidae), which may coincide with the fragmentation of forest habitat and the isolation of ancestral mangabey populations into diverging lineages.

Intraspecific competition: Cercopithecus and Papio

Finally, intraspecific competition may have further influenced dispersal and isolation alongside physical barriers. The current and ancient widespread *Cercopithecus* species are thought to have influenced the relictual distribution of the *Cercocebus* species through competition (Butynski et al. 2013). The historic dispersal of *Cercopithecus* sp. in East Africa is thought to have also been influenced by the Rift Valley and increasing unsuitable arid habitat (Butynski and Jong 2020). The expansion of *Cercopithecus* (*nictitans*) species diversity was estimated to have occurred 2.1 – 2.4 MYA (Butynski and Jong 2020) coinciding with the divergence time (2.34 MYA) of the Sanje mangabey from other *Cercocebus* species. This diversification and the expansion and contraction of forest habitats may have led to direct competition between the ancestral Central/Eastern *Cercocebus* populations with *Cercopithecus* sp. (e.g. ancestral populations of *Cercopithecus mitis* found in the Udzungwa Mountains), resulting in the isolation of populations, prevention of dispersal between contracting intermediate forests, and possibly leading to the extinction of *Cercocebus* populations. It is likely also the Sanje mangabey experienced competition with other semi-terrestrial primates or were isolated due to avoidance of other species such as the yellow baboon (*P. cynocephalus*) that are well-adapted to the lower elevation forests and savannas. Currently the mangabeys avoid interactions with the baboons (McCabe and Fernández, pers. obs.). Tana River mangabeys have also been observed avoiding yellow baboon, where mangabeys avoided regions of habitat overlap between the two species, reducing their within-group dispersal when habitat overlap was high with baboons and being displaced in sleeping sites (Wahungu 1998). These behaviours reduce habitat overlap between the species, possibly leading to low competition. However, the behaviours were observed in a period of high fruit availability, which reduced the need for overlap with abundant resources available across the habitat area. In periods of reduced food resources, both species would occupy the same habitats, potentially increasing competition and likely to the detriment of the mangabeys (Wahungu 1998). Therefore, it is possible that avoidance and competition for resources between mangabeys and other species limited the dispersal between forest habitats at a time the forests were becoming smaller and fewer, isolating mangabey populations.

In order to further understand the relationship between the *Cercocebus* and *Mandrillus* species it would be beneficial to undertake extensive sampling from across the current known distribution for each species. This would identify whether isolated populations hold cryptic diversity that may provide insight into the dispersal routes by identifying time to most recent common ancestor at both the population and species level. This study also used only four markers, therefore, genome-wide markers combined with behavioural and morphological studies would provide a more accurate estimate of relationships between species.

3.4.2. Ancient Lineages

The two Sanje mangabey populations had an estimated ancient divergence time for the concatenated mitochondrial and nuclear sequences. In this study, the TMRCA for the two Sanje mangabey populations was an estimated 0.77 MYA (95% CI: 0.43 – 1.15). A previous estimate from Struhsaker et al. (Struhsaker et al. 2004) suggested that the forests separated over 100 years ago, however this result, alongside other studies of genetic structure within the Udzungwa Mountains forests (East Afromontane horned chameleons, *Triceros* sp. (Ceccarelli et al. 2014); grey-faced sengi, *Rhynchocyon udzungwensis* (Lawson et al. 2013), suggest that the division of subpopulations across taxa within the forest fragments occurred on a more ancient timescale. The divergence time found in this study suggests the two subpopulations are representative of two ancient lineages, rather than becoming isolated with more recent anthropogenic activity. As with the isolation of the Sanje mangabey from other *Cercocebus* mangabeys, this ancient isolation of the two subpopulations to relatively distant (~100km) forest blocks may be the result of a drying climate in the region and physical barriers, coupled with interspecific competition and avoidance from other terrestrial primate species preventing dispersal between forest blocks. This suggests that the two subpopulations may represent evolutionary distinct lineages. This result is an example of the need to study population level genetic structure to identify cryptic diversity within species, particularly with conservation efforts in mind. Further study of the Sanje mangabey subpopulation genetic structure is ongoing using a larger number of samples, and mitochondrial DNA and genome-wide nuclear DNA.

3.4.3. Conclusions

This study suggests that the Sanje mangabey lineage is evolutionarily distinct and therefore warrants conservation protection as a distinct management unit from other mangabeys. In addition, the Sanje mangabey populations should be considered candidate evolutionary significant units (Moritz 1994) and conservation management planning must now take this into consideration. This estimated time of divergence between populations has conservation implications, and further morphological, molecular, and behavioural study is required to determine how reproductive isolation has shaped these features. If conservation actions such as genetic rescue, translocation or captive breeding are to be considered, the risk of outbreeding depression between populations should be estimated prior to actioning.

3.5. ACKNOWLEDGEMENTS

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CHAPTER FOUR

Phylogeography and historical niche modelling reveals the evolutionary history of the Endangered Sanje mangabey (*Cercocebus sanjei*)

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ABSTRACT

The Sanje mangabey (*Cercocebus sanjei*) is an Endangered primate endemic to the Udzungwa Mountains, south-central Tanzania. The species is divided into two populations that are isolated to two forest situated 100km apart. The two extant populations represent two distinct evolutionary lineages that diverged 0.77 million years ago (MYA). This study investigated the genetic diversity and the phylogeographic history of the two Sanje mangabey populations and recent changes in size and range. We estimated mitochondrial DNA (mtDNA) diversity using a 423bp fragment from the mitochondrial control region (Mwanihana: n=36; Uzungwa Scarp: n=28) and describe diversity through summary statistics and a haplotype network. The demographic history of the populations is estimated using a mutation-scaled effective population size to estimate female effective population size, and estimating a growth rate for each population. Further, the probability of suitable habitat across Tanzania and the Udzungwa Mountains is modelled using bioclimatic variable layers. The current known distribution was used to identify regions estimated to have a high probability of presence for the Sanje mangabey at three time points: Mid-Holocene (6,000 YA), Last Glacial Maximum (22,000 YA), and the Last Interglacial period (120,000-140,000 YA). Twenty-two segregating sites and six haplotypes (Mwanihana: n=2; Uzungwa Scarp: n=4) were found across all mitochondrial control region sequences. Two haplogroups were found; these were separated by a minimum of 16 substitutions and were structured by the two populations. In Mwanihana, 35 samples comprised a single haplotype, differing by one substitution to a second haplotype harboured by only one individual. Haplotype diversity, nucleotide diversity, and female effective population size was higher in the Uzungwa Scarp, with four haplotypes present and five segregating sites. Significant differentiation was estimated between populations (F_{ST}=0.95, p<0.001). Estimates for Tajima's *D* and Fu's F_s within each population showed no evidence for recent expansion or contraction, and the estimated growth rate suggested female effective population sizes to have been stable. Ecological niche modelling revealed fluctuating extents of suitable habitat with environmental change larger than the present day in the ~6,000 YA model across southern Tanzania, but with similar extent to the present in the ~22,000 YA model, and then still smaller during much less suitable conditions across the Udzungwa Mountains and Tanzania ~120,000 – 140,000 YA. The significant differentiation between populations may have been influenced by a general trend in aridification in East Africa over this time, resulting in a shift of montane forests to gradually higher elevations over the last 40,000 years. With fluctuations over this period driving habitat fragmentation and isolating populations, intermediate populations may have become extinct as suitable habitat retracted, leaving relict populations with relatively stable demographic histories ancestral to the present-day populations. Riverine barriers, interspecific competition and avoidance competition may have also limited dispersal between these fluctuating habitats over time. This significant differentiation between populations supports their preliminary designation as separate evolutionary significant units.

4.1. INTRODUCTION

The Sanje mangabey (*Cercocebus sanjei*) is an Endangered primate that is endemic to the Udzungwa Mountains, Tanzania (McCabe et al. 2019). The species is only found in two forest fragments: Mwanihana, in the Udzungwa Mountains National Park, and the Uzungwa Scarp Nature Reserve. The Uzungwa Scarp is currently isolated and no viable ecological corridor exists between the Uzungwa Scarp and neighbouring forests (WWF Tanzania Programme Office 2007). The two forests in which the populations are found are ~100 km apart, and are separated by habitats thought to be unsuitable for the Sanje mangabey (Rovero et al. 2014).

This division into two small and isolated populations suggests that the species is at great risk of potentially negative stochastic demographic and genetic processes, such as genetic drift and inbreeding depression, and loss of genetic diversity and adaptive potential (Frankham 2010). It is currently unknown when these two forests became isolated, with one study suggesting *'some have probably been separated for >100 years'* (Struhsaker et al. 2004). From a phylogenetic study of the Sanje mangabey using two nuclear and two mitochondrial markers, the two Sanje mangabey populations were estimated to be representative of two evolutionary distinct lineages (Chapter Three). The lineages were estimated to have diverged 0.77 million years ago (MYA) and were suggested to be considered preliminary evolutionarily significant units pending further study (Paddock et al., in prep; Chapter Three).

No previous studies have been conducted to determine the phylogeographic structure of the Sanje mangabey populations to investigate the underlying causes of the genetic divergence found for the two populations. However, as with the divergence time estimated between populations, evidence from recent genetic studies of other animals in the Udzungwa Mountains have found significant genetic differentiation between forest blocks with isolation of fragments indicating a relatively ancient timescale (Struhsaker et al. 2004). One study found evidence for mitochondrial DNA introgression between some populations of the grey-faced sengi (*Rhynchocyon udzungwensis*) and the parapatric and more widespread chequered sengi (*R. cirnei reichardi*; Lawson et al. 2013). However, nuclear loci showed clear monophyly between species, suggesting introgression occurred on a historical timescale, coinciding with interglacial climate cycles altering each species' distribution (Lawson et al. 2013).

A recent study of the landscape genetics of the sympatric and endemic Udzungwa red colobus (*Piliocolobus gordonorum*) across five forests (including Mwanihana and the Udzungwa Scarp) found that, although neutral genetic diversity did not differ within fragments, there was genetic differentiation between fragments (Ruiz-Lopez et al. 2016). They also found that variables, such as proximity to human settlements and fire density (mostly human set fires that threaten the forest; Dinesen et al. 2001), were shaping the species' genetic structure. Nevertheless, results suggested that genetic differentiation may still be a product of ancient fragmentation and recent human activity was responsible for maintaining existing barriers to dispersal (Ruiz-Lopez et al. 2016). The Uzungwa Scarp population was the most isolated population with the highest differentiation from other populations, highest number of unique alleles and lack of migration for many generations, and this is thought to be an indicator of the patterns found in other species in the forests.

The Sanje mangabey populations are estimated to occupy an elevation range between 290 – 2000 m a.s.l. (McCabe et al. 2019). The extant populations are flexible in their habitat use, successfully making use of primary and secondary forest, and elephant-disturbed shrubland (McCabe et al. 2013). Throughout the Udzungwa Mountains other forests exist within this elevation range with similar habitat composition. Further these forests are inhabited by primate species that are sympatric with the Sanje mangabey in Mwanihana and the Uzungwa Scarp (Rovero and de Luca 2007). It is likely that ancient Sanje mangabey subpopulations existed in other forest blocks within the region that have since become extinct, with the original description of the species suggesting this may have included Kilombero Valley lowland forests (Homewood and Rodgers 1981), an area located south of both extant populations along the River Kilombero (Figure 4.1). This extended range may have included two neighbouring forests to Mwanihana (<30 km): Nyanganje (41.9 km^2; 350-1,038 m) and Luhombero/Ndundulu (230.6 km^2; 1,105-2,520 m), both home to the Udzungwa red colobus, black-and-white colobus (*Colobus angolensis palliatus*) and the Syke's monkey (*Cercopithecus mitis*), with the yellow baboon (*Papio cynocephalus*) and vervet monkey (*Cercopithecus aethiops*) also present in Nyanganje and the kipunji in Luhombero (Figure 4.1). These forests are considered to have low human impact with relatively few communities in these regions (Marshall 2007), but have previously been targets for logging (Dinesen et al. 2001; Rovero and Marshall 2005) and poaching (Jones et al. 2005). Similarly, neighbouring the Uzungwa Scarp (<30 km), forests within this elevation range include Kisinga-Rugaro (116.2 km^2; 1,627-2,322 m) and New Dabaga/Ulangambi (40 km^2; 1,764-2,081 m), with the black-and-white colobus, Syke's monkey, and vervet monkey present in both forests, and the Udzungwa red colobus in New Dabaga. No endemic species of primate are found in Kisinga-Rugaro; however, the Udzungwa red colobus has been reported to have been present previously (Lovett and Pócs 1993). Both of these forests have been subject to high anthropogenic activity, especially extensive logging in the 1970s (Dinesen et al. 2001). The Sanje mangabey, therefore, could have inhabited these forests and become extirpated with habitat degradation and indirectly from poaching (e.g., mangabeys are often caught in snares set for other terrestrial mammals; Rovero et al. 2009).

Ecological niche modelling (ENM) can provide insight into the suitability of areas not currently occupied by the Sanje mangabey but which are similar to the ones at Mwanihana and the Udzungwa Scarp. ENM is a mathematical tool used to estimate the probable area of suitable habitat and a predicted distribution can be modelled using current presence data and environmental parameters. Moreover, combining estimated paleodistributions, projected from historic bioclimatic estimates, with genetic structure from current sampling can provide a spatial perspective on a species' demographic history and distribution. ENM is an invaluable tool for conservation as it can provide information on the importance of environmental variables and potential distribution of species to target surveys and potential areas for conservation (Fitzgerald et al. 2018; Liu et al. 2019), and assist in optimal land use planning to preserve biodiversity by local stakeholders (Gregory et al. 2012; Zhang et al. 2012).

Determining whether the current distribution of a species is a function of recent and/or anthropogenic-related events, versus more ancient and vicariant factors, is important for the creation of appropriate conservation policies (Radespiel and Bruford 2014). Significant divergence in genetic structure between populations would substantiate a claim for those populations to be classified as: i) separate management units (MUs), if significantly differentiated in the frequency of either mitochondrial (mtDNA) or nuclear DNA markers, or ii) as evolutionarily significant units (ESUs) where reciprocal monophyly is found for both mtDNA and nuclear DNA (Moritz 1994). The identification of such units should then be incorporated in conservation action plans to ensure genetic diversity is adequately protected and included in assessments of measures such as taxonomic designation, translocation, and landscape restoration. For example, a study using a fragment of the mtDNA control region identified two ESUs in muriquis in the Brazilian Atlantic Forest (northern: *Brachyteles arachnoides*; and, southern: *B. hypoxanthus*; Chaves et al. 2019). Therefore conservation strategies in this study were tailored for each population to preserve the ESUs, rather than following the monotypic classification *B. arachnoides* and applying the same actions to both populations (Chaves et al. 2019).

MtDNA is a marker that has been used since the 1980s and is still frequently used in current population genetic and phylogenetic studies. This long-term use of the marker has resulted in a large database of sequences for a wide range of species. When using non-invasively collected samples (e.g., hair or faeces), mtDNA is advantageous as it is easily amplified with PCR (due to multiple copies within each cell) compared to nuclear DNA. The maternal evolutionary history can be inferred by using mtDNA as it is maternally inherited, has a negligible recombination rate (with the exception of rare paternal leakage; White et al. 2008), and haploid inheritance, which also means the whole mitochondrial genome acts as a single locus. Different regions within the mtDNA genome exhibit varying mutation rates, with the non-coding control region and especially the 'D-loop' in primates the fastest evolving region (Moritz et al. 1987; Subramanian and Lambert 2011).. This faster evolution means that more recent changes are better detected using mitochondrial DNA than nuclear. With only maternal inheritance, the effective population size for mtDNA is far smaller than autosomal DNA (approximately 25%), meaning it is more affected by processes such as genetic drift. The combination of this fast mutation rate and random genetic drift makes mtDNA a suitable marker for barcode identification of species, studying recent and historic phylogeographic patterns, and constructing phylogenies (Sunnucks 2000).

4.1.1. Aims and Hypotheses

This study aimed to characterise the phylogeographic structure, mitochondrial genetic diversity, and recent demographic history of the Sanje mangabey using a fragment of the mtDNA control region, amplified from samples collected from across the distribution of both populations. We used ecological niche modelling to estimate the extent, location, and temporal dynamics of possible suitable habitat for the Sanje mangabey over the last 140,000 years. This information was used to assess whether lack of suitable habitat and consequent inability to disperse between populations may have impacted on the 0.77 MY divergence time (Chapter Three). We hypothesised that the phylogeographic structure of the Sanje mangabey populations would reflect the ancient timescale of divergence (0.77 MYA; Chapter Three) and therefore would show significant mitochondrial differentiation between populations, reflecting evolutionarily distinct lineages requiring independent conservation action. Further, combining the demographic history with the ecological niche modelling, we hypothesised that patterns in effective population size would follow trends of estimated suitable habitat extent. Conservation management recommendations based on results from genetic data analyses and ENMs are included, with the objective to complement the *Mangadrill Conservation Action Plan* currently under development (Fernández et al. 2019).

4.2. METHODS

4.2.1. Mitochondrial DNA Phylogeography

The Sanje mangabey is present in two forests within the Udzungwa Mountains: Mwanihana (7°40’-7°57’S, 36°46’-36°56’E) within the Udzungwa Mountains National Park, and the Uzungwa Scarp Nature Reserve (8°14’-8°32’S, 35°51’-36°02’E; Figure 4.1). Faecal samples were collected opportunistically from unidentified and non-observed individuals across 28 unique locations, coinciding with a population survey of the species within both forests (Paddock et al. 2020; Figure 4.1).

Faecal samples were collected whilst following groups located from vocalisations. Samples were collected only if they were 2 m from another sample to minimise the probability of repeatedly sampling an individual. Samples were stored following the two-step method described by Roeder et al. (2004)Roeder et al. (2004). DNA was extracted from faecal samples using material taken from the outer surface and a QIAamp Fast DNA Stool Mini Kit (Qiagen, UK) following a modified manufacturer’s protocol (see Chapter Three). Samples used were selected to represent multiple individuals at each unique sampling location, randomly choosing samples from each location. Extractions from a total of 64 samples were used in this study: 36 from Mwanihana and 28 from Uzungwa Scarp.

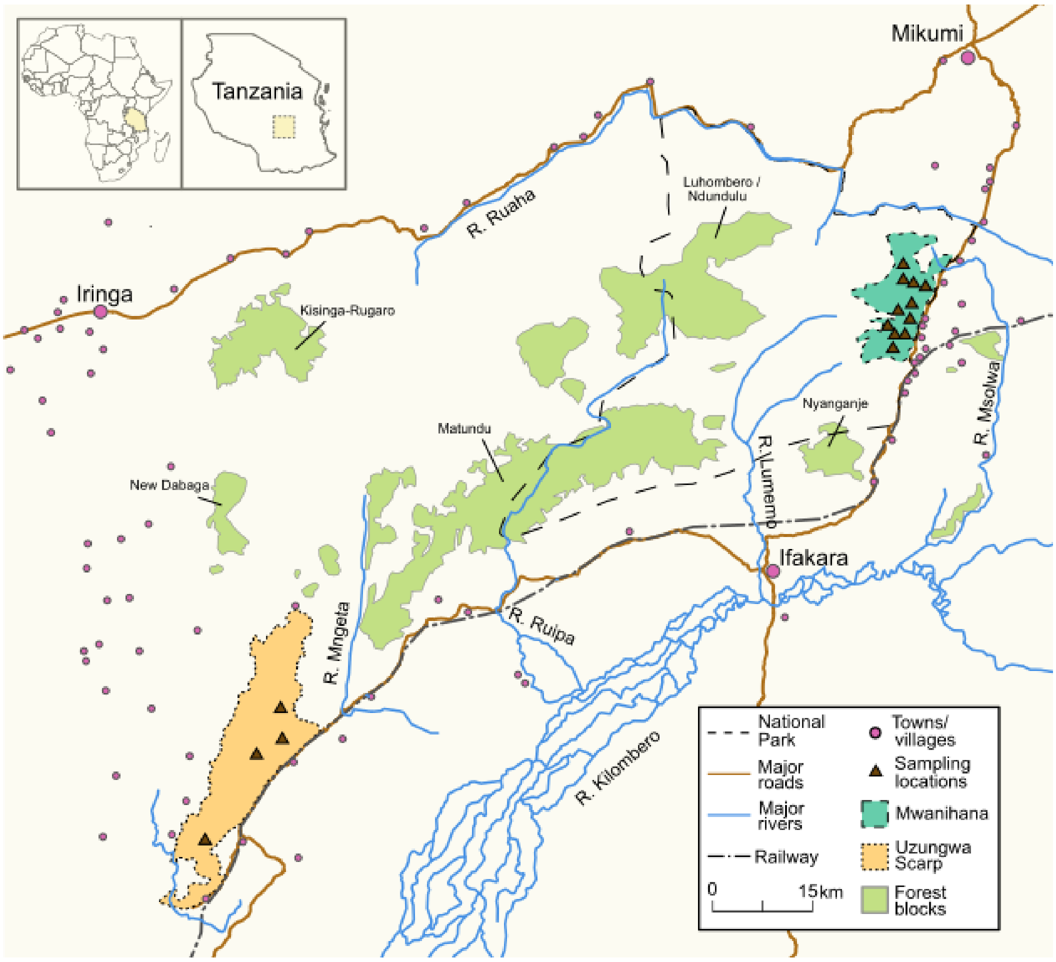


Figure 4.1. A map of the Udzungwa Mountains, Tanzania showing the two forests where the Sanje mangabey is found: Mwanihana forest and the Uzungwa Scarp. The locations where faecal samples were collected are highlighted.

After DNA extraction a ~580 bp fragment of the mtDNA CR spanning sections of both the hypervariable regions I and II (HVRI: ~350 bp; HVRII: ~230 bp) was amplified using sequences from two overlapping primers (*Cercocebus atys* KP090062.1: 15,637 – 16,214; Table 4.1). Each fragment was amplified by Polymerase Chain Reaction (PCR; conditions in Table 4.1) in a total volume of 10 μl, consisting of 5 μl Bioline MyTaq™, 1 μl template DNA and forward and reverse primers at a final concentration of 2 μM. All PCR cycling programmes were run in an Applied Biosystems GeneAmp PCR System 9700. The success of each PCR was tested by agarose gel electrophoresis and visualised in a UV transilluminator, and PCR products sent for bi-directional sanger sequencing using either the Eurofins PlateSeq Service (Ebersberg, Germany) and Centre for Molecular Analysis sequencing service (CTM; CIBIO, Portugal). The chromatogram for each sequence was inspected manually using Geneious (v4.8.5) to trim forward and reverse ends and verify the quality of base calls. Sequences were trimmed to the length of the shortest sequence for an individual to minimise missing data between Sanje mangabey samples, resulting in a 423 bp fragment for analyses. Each sequence was submitted to BLAST (Basic Local Alignment Search Tool; https://blast.ncbi.nlm.nih.gov/Blast.cgi) to search against existing sequences in GenBank and only samples where the percentage identity top results (>95%) were *Cercocebus* sequences and were included in further analyses (sequences removed discussed in Chapter Three).

Table 4.1. Primer sequences to amplify two overlapping fragments of the mitochondrial control region for the Sanje mangabey (*Cercocebus sanjei*) from Chapter Three.

Primers		**Fragment length (bp)**	**PCR Cycling Conditions**
CRF-1	GCTCCGGGCCCATAACTC	257	95°C for 2 mins, [95°C for 30s, 61.8°C for 60s, 72°C for 2 mins] x 35 cycles, 72°C for 10 mins
CRR-1	CAAAGACAGGCGCATTCAGG		
CRF-2	CCRAAACATGCTTACAAGC	343	95°C for 2 mins, [95°C for 30s, 50°C for 60s, 72°C for 2 mins] x 35 cycles, 72°C for 10 mins
CRR-2	GTTATGGCCCTGAGGTAAG		

Following (Bensasson et al. 2001), several precautions were taken to avoid and verify the co-amplification of nuclear mitochondrial inserts (NUMTS): i) sequence chromatograms were inspected for double peaks that would indicate the presence of sequences co-amplified by PCR and which could represent contaminations or NUMTS, ii) only a single PCR amplicon of the correct length was present, iii) any single base extreme variants found during the alignment of sequences were verified, and iv) four independent primers amplifying overlapping fragments were used, with the overlapping sequences matching from separate PCRs (Table 4.1).

The number of polymorphic sites, number of haplotypes, haplotype diversity and nucleotide diversity were estimated using DnaSP v 6.12.01 (Rozas et al. 2017). A TCS network of haplotypes was built using PopART v1.7 to estimate the relationship between haplotypes Leigh and Bryant 2015). The genetic structure of the population was tested using an Analysis of Molecular Variance (AMOVA) and the fixation index (F_{ST}), computed using pairwise differences with 10,000 permutations in ARLEQUIN 3.5.2.2 Excoffier and Lischer 2010).

The inference of the demographic history when differentiated populations are analysed together as a single unit may produce an erroneous signal of a demographic change (Heller et al. 2013). Thus, as populations were found to be representative of ancient lineages previously (Chapter Three) and significant differentiation was found between populations in this study (see Results), the demographic history was inferred for the two populations independently. To investigate the demographic history of the populations, neutrality tests Tajima's *D* (distribution of segregating sites) and Fu's F_s (haplotype distribution) statistics were carried out in ARLEQUIN 3.5.2.2 (Excoffier and Lischer 2010) using 10,000 simulations for each population individually. Positive values of *D* and F_s would indicate the loss of rare alleles following population contractions, whereas significant negative values would indicate a recent population expansion. Statistical significance was considered at $p<0.05$. Additionally, the growth rate (g), to further estimate recent expansion or decline, and the mutation-scaled effective population size (Θ) was estimated using LAMARC 2.1.10 (Kuhner 2006). The F84 model was used (to reflect the HKY substitution model determined to be most suitable) with a Bayesian search strategy. The initial chains were run for 10 chains of 4,000 steps, with the first 400 steps discarded as a 10% burn-in, to estimate reasonable parameter likelihoods to inform the final chains. The final chains were run for five chains of 400,000 steps, with the first 4,000 steps discarded as burn-in. Using the estimations of mutation-scaled effective population size (Θ) and the mutation rate/site/generation (μ), we estimated the female effective population size (N_{ef}) using the formula $N_{ef} = \Theta/2\mu$. We estimated μ using the mutation rate for the mtDNA control region HVRI estimated for African apes and humans (1.64×10^{-7} substitutions per nucleotide per year; Soares et al. 2009) and the generation time for the mangabeys of 19 years (Ehardt et al. 2005).

4.2.2. Ecological Niche Modelling

Ecological niche modelling maximum entropy framework, a model that minimises the relative entropy between probability densities from presence data and environmental data (see Elith et al. 2011), was used to calculate the probable distribution of the Sanje mangabey using the software Maxent v 3.4.1 (Phillips et al. 2006) across Tanzania. Only presence data was used, collated from geographic coordinates of vocalisations detected during a population survey (Paddock et al. 2020). To remove duplicate records and spatial autocorrelation, the presence points were filtered by placing a 2.5 minute grid on each forest, reflecting the largest cell size from the downloaded bioclimatic layers, and randomly selecting a single presence point from each cell using R Version 3.5.2 (R Core Team 2018). This filtered an original 856 presence co-ordinates to 18 presence points (Mwanihana: n=10; Uzungwa Scarp: n=8) that were used as the occurrence points in the Maxent model.

Nineteen 'near current' (1960-1990) bioclimatic variables were downloaded from WorldClim Version 1.4 (http://worldclim.org/) at 30 second resolution (aiming for the highest resolution available at the time of this study; Table 4.2). Layers were cropped to the same extent, creating a study area within the boundaries of 27.5-42°E, 0-12°S, to estimate areas of probable occurrence across Tanzania. Layers were projected to the same co-ordinate reference system (WGS84:4326) and resampled to 30 s using the *raster* package in R (R Core Team 2018). A Pearson's correlation (*r*) test was used in R (R Core Team 2018) to measure the correlation between variables. Where variables were highly correlated ($-0.7 < r > 0.7$), one variable was retained prioritising the broader variable (i.e. annual mean temperature over specific seasonal variables). This resulted in 13 of the 19 layers being removed from the analyses (Table 4.2). The remaining six variables as estimated for three paleo-bioclimatic scenarios were downloaded from WorldClim 1.4 and used for projections of estimated suitable environmental conditions. This included the Mid Holocene (MH: ~6,000 YA; 30 secs), the Last Glacial Maximum (LGM: ~22,000 YA; 2.5 mins), and the Last Inter-Glacial (LIG: ~120,000 – 140,000 YA; 30 secs; (Otto-Bliesner et al. 2006).

Table 4.2. Current bioclimatic variables (1960-1990) from WorldClim Version 1.4 (http://worldclim.org) analysed during ecological niche modelling for the Sanje mangabey in the Udzungwa Mountains, Tanzania. Variables used in the model after testing for autocorrelation are indicated in the final column.

Variable	Description	
Bio1	Annual mean temperature	✓
Bio2	Mean diurnal range: (Mean max. temp – min. temp)	✓
Bio3	Isothermality: temperature variability from day to night and between seasons - (Bio2/Bio7) *100	✓
Bio4	Temperature seasonality: (Standard Deviation * 100)	
Bio5	Max. temperature warmest month	
Bio6	Min. temperature coldest month	
Bio7	Temperature annual range: (Bio5 – Bio6)	
Bio8	Mean temperature of wettest quarter	
Bio9	Mean temperature of driest quarter	
Bio10	Mean temperature of warmest quarter	
Bio11	Mean temperature of coldest quarter	
Bio12	Annual precipitation	✓
Bio13	Precipitation of wettest month	
Bio14	Precipitation of driest month	✓
Bio15	Precipitation seasonality	
Bio16	Precipitation of wettest quarter	
Bio17	Precipitation of driest quarter	
Bio18	Precipitation of warmest quarter	✓
Bio19	Precipitation of coldest quarter	

To estimate the best fitting model, the ENMeval package (Muscarella et al. 2018) in R (R Core Team 2018) was used to model combinations of the regularisation multiplier and feature classes. The following combinations of the five feature classes (linear: L, quadratic: Q, product: P, hinge: H, and threshold: T) were used: 'L', 'LQ', 'H', 'LQH', 'LQHP' and 'LQHPT' and ten regularisation multiplier values (1-10). The best model would be estimated where ΔAIC = 0. The model's Area Under the Receiver Operating Characteristic (AUC) curve was estimated as a measure of the model performance. An AUC value of 0.5 for a model indicates random prediction and values closer to 1 indicate better model performance. The AUC in a presence-only model indicates whether the probability values of the model estimated for presence points are higher than those for the values the background points. The model, with the features and regularisation multiplier determined to be most appropriate, was run using the cross-validation replicate run type for 25 replicates, and with a maximum of 10,000 background points. The cross-validation replicate method is best for small datasets as it uses all the data for validation in a series of 'folds' with the data split into equal size groups (Phillips 2017). The Maxent parameters were set to logistic output and 5,000 iterations for each simulation.

Response curves were generated for the current model to estimate the contribution of each predictor variable on the probability of presence. Jack-knife of regularised training gain (the estimated predictive power) was estimated for each predictor variable to estimate the contribution of each parameter to presence singularly, when acting as the only variable (training gain) and when individually removed from the full model (permutation importance). To determine probable habitat suitability, a minimum training presence threshold (MTP) was used. In this method, the values from areas estimated to be the lowest probability of suitable conditions set the minimum threshold level. This threshold method was chosen as a conservative method for estimating habitat suitability, using the data within the model to establish the thresholds, rather than using fixed subjective values (Liu et al. 2005).

4.3. RESULTS

4.3.1. Mitochondrial Phylogeographic History

Sixty-four Sanje mangabey mtDNA control region sequences were used. A total of 22 segregating sites (all transition substitutions) and six haplotypes were found (Table 4.3). No evidence of NUMTS was found in the sequences following recommendations from Bensasson et al. (2001) and the presence of only transition substitutions further supports a non-nuclear source for the sequences (Brown et al. 1982). Haplotype and nucleotide diversity were higher in the Uzungwa Scarp than Mwanihana (Table 4.3) with two haplotypes found in Mwanihana and one segregating site; and four haplotypes in Uzungwa Scarp, with five segregating sites (Table 4.3). The haplotype network showed a frequent and central haplotype for Mwanihana, connected by one mutation difference to a rare haplotype, harboured by one individual (Figure 4.2). For Uzungwa Scarp, the network pattern showed two more frequent haplotypes, connected by three mutation differences with no clear central structure, with one of these haplotypes connected to two less frequent haplotypes by one substitution to each (Figure 4.2). No shared haplotypes were found between the two populations. The two haplogroups, represented by Mwanihana and Uzungwa Scarp, were separated by 16 substitutions. The populations were found to be significantly differentiated with nucleotide variation found to be 95.49% among populations, and only 4.51% within populations (F_{ST}=0.95, p<0.001; Table 4.4).

Using Tajima's D and Fu's Fs, no significant evidence for demographic expansion or contraction was found for Mwanihana (n=36 sequences: D = -1.33, p>0.10; F_S = -1.36, p>0.10) or the Uzungwa Scarp (n=28 sequences: D = 1.04, p>0.10; F_S = 1.914, p>0.10). The mutation scaled effective population size (Θ) estimates showed a difference between the populations with Mwanihana (Θ = 0.0006; 95% CI: 0.00002 – 0.0034) estimated to be lower than Uzungwa Scarp (Θ = 0.0027; 95% CI: 0.00077 – 0.0098). The female effective population size was estimated to be considerably lower for Mwanihana (N_{ef} = 96; 95% CI: 3 – 546) than in the Uzungwa Scarp (N_{ef} = 433; 95% CI: 124 – 1573). The estimated growth rate for each population showed a positive value (Mwanihana: 882, 95% CI: -455 – 970; Uzungwa Scarp: 4.69, 95% CI: -455 – 938). However, the 95% confidence interval for both populations was wide and the lower range were negative values suggesting a low likelihood of population expansion.

Table 4.3. Population mitochondrial genetic diversity indices for each Sanje mangabey (*Cercocebus sanjei*) for each population individually and for the total population, using a 423 bp fragment of the control region.

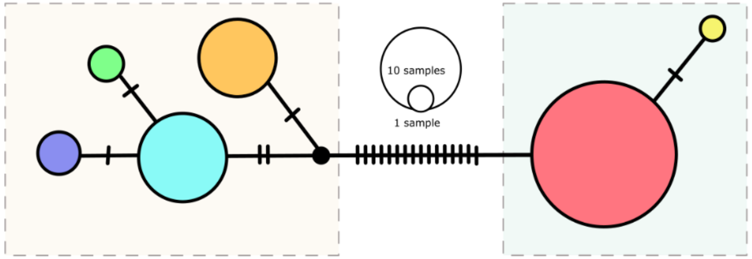
n = sample size; N_{hap} = number of haplotypes; *h* = haplotype diversity, *S* = polymorphic sites;
π = nucleotide diversity; k = mean pairwise differences.

Population	***n***	N_{hap}	***h* (±SD)**	***S***	**π (±SD)**	**k**
Mwanihana	36	2	0.056 (±0.052)	1	0.00013 (±0.00012)	0.056
Uzungwa Scarp	28	4	0.664 (±0.055)	5	0.00417 (±0.00041)	1.756
ALL	64	6	0.642 (±0.051)	22	0.022 (±0.011)	9.273

Table 4.4. Analysis of Molecular Variance (AMOVA) of genetic differentiation among and within the two Sanje mangabey (*Cercocebus sanjei*) populations for both haplotypic and locus-by-locus distance matrices.

Sources of variation	**d.f.**	**Sum of squares**	**Variance components**	**Percentage of variation**
Among populations	1	267.30	8.47	95.49
Within populations	62	24.79	0.40	4.51
Total	63	292.09	8.87	

a) Haplotype Network



b) Haplotype Location

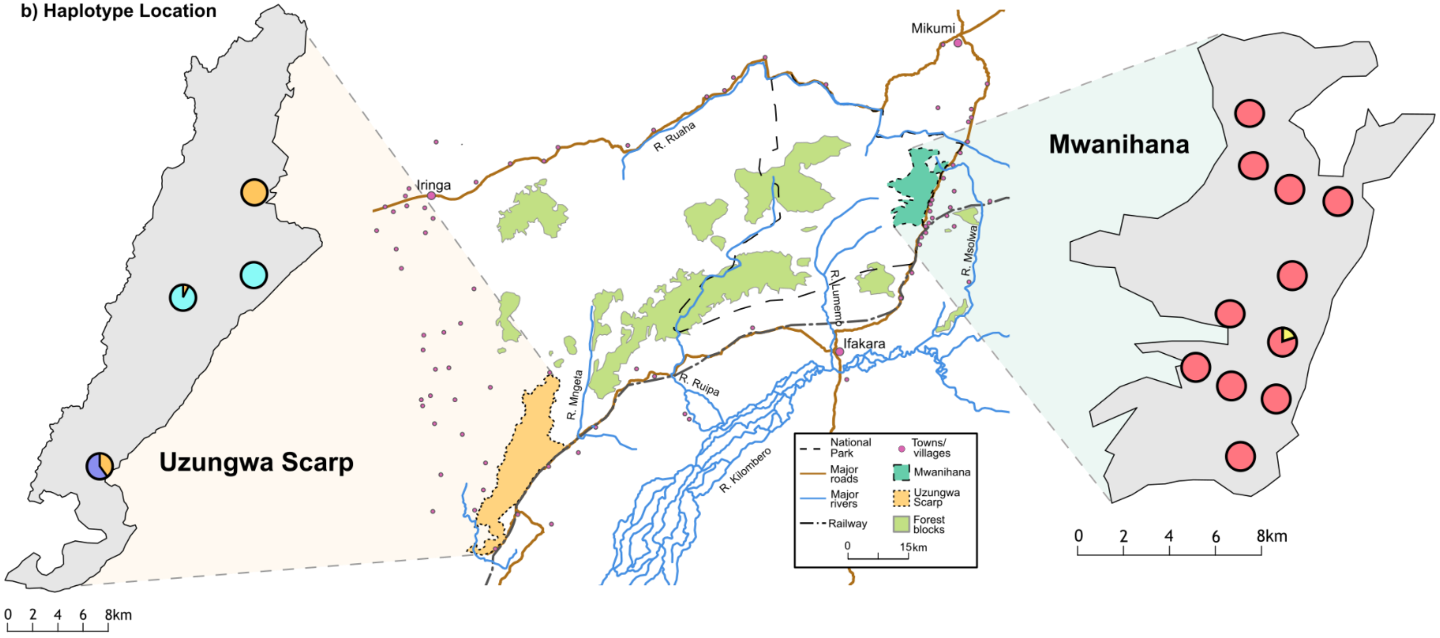


Figure 4.2. Genetic diversity and phylogeographic structure inferred using a 423 bp fragment of the mitochondrial control region fragment for the Sanje mangabey (*Cercocebus sanjei*) across the forests blocks in the Udzungwa Mountains, Tanzania: a) the TCS haplotype network showing the relationship between the six haplotypes that were found from 64 sequences; two in Mwanihana (n = 36) and four in the Uzungwa Scarp (n = 28) and b) the location of the haplotypes. The colours of haplotypes correspond between each figure and circles are proportional to the frequency of each haplotype in a) the size of the circle and in b) the proportion of the circle.

4.3.2. Ecological Niche Modelling

Model evaluation estimated an optimal solution using linear, quadratic and hinge feature classes ('LQH') and a regularisation multiplier of 4 to be the best fitting model (AICc = 451.89; ΔAIC = 0). The mean AUC of the Maxent model from averaging the results from 25 runs using the cross-validation method was 0.976 ± 0.012, which indicated the data fitted closely with the estimated distribution. The variables with the greatest contribution to the model were isothermality (bio3; 38.8%), the precipitation during the driest month (bio14; 38.5%), and annual precipitation (bio12; 19.3%; Figure 4.3; Table 4.5). The variable for precipitation of the driest month (bio14) had the greatest training gain when used by itself within the model and the overall model training gain decreased most when it was removed, indicating that the variable contains the most information not available in other variables (Figure 4.3). It was estimated that precipitation of the driest month had the greatest permutation importance (bio14; 52.4%), followed by isothermality (bio3; 36.4%). The other variables had lower estimated importance (Table 4.5).

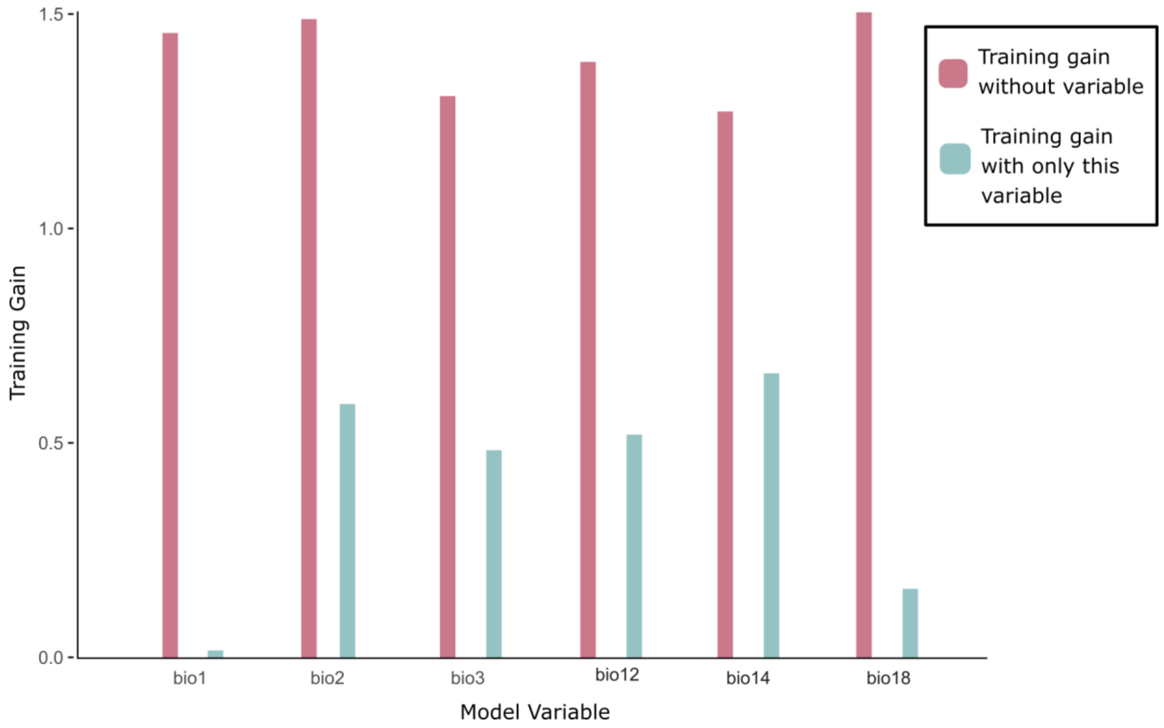


Figure 4.3. Jack-knife test of importance for each variable by testing the training gain of the model (i.e. its predictive power). The graph shows for each variable, the difference in average gain built without a given variable (red) and models built with only that variable (green) using training and test data. The variables with smaller differences are ones most related to species distribution.

Table 4.5. The estimates of contributions to the Maxent model by each variable.

Variable		Contribution (%)	Permutation importance
bio3	Isothermality	38.8	36.4
bio14	Precipitation during the driest month	38.5	52.4
bio12	Annual precipitation	19.3	5.5
bio1	Annual mean temperature	2.1	4.5
bio2	Mean diurnal range	1.2	1.2
bio18	Precipitation of warmest quarter	0	0

For habitat suitability and presence probability to be estimated over 0.7 (high suitability) in the response curves for the top three contributing variables, isothermality was in the lower range of values in the model (<63; Figure 4.4a), a moderate range of precipitation of the driest month (~3-11 mm; Figure 4.4b), and high annual precipitation (>1300mm; Figure 4.4c). In the present day scenario, the estimated suitable habitat and probability of presence was high (>0.7) across southern Tanzania, extending from the Southern Highlands across to the southern region of the Udzungwa Mountains (Figure 4.5). In the Mid-Holocene (~6,000 YA), the estimated suitable habitat was larger than the current scenario, extending over most of south Tanzania and covering much of the Udzungwa Mountain range (Figure 4.6). During the Last Glacial Maximum (~22,000 YA), the estimated suitable habitat was smaller compared to that shown in the mid-Holocene, resembling more the current day habitat suitability however high probability regions extended further south from the Southern Highlands (Figure 4.7). Finally, in the Last Interglacial period (~120,000 – 140,000 YA), the estimated suitable habitat was even smaller, with fragments of suitable habitat north-west of the Tanzanian border and Lake Tanganyika, in the Southern Highlands and south of the Udzungwa Mountains in the Kilombero Valley (Figure 4.8).

4.4. DISCUSSION

This study provides the first evidence of the phylogeographic structure of the Sanje mangabey and its two populations and estimates the area of suitable habitat for the present day, Mid-Holocene, Last Glacial Maximum, and Last Interglacial periods. The study also investigated the genetic structuring of the two Sanje mangabey lineages described in Chapter Three. Our results suggested the current populations are descended from ancient lineages that diverged 0.77 MYA. Additionally, using ecological niche modelling, we identify those factors most likely to contribute to the isolation of the two population within

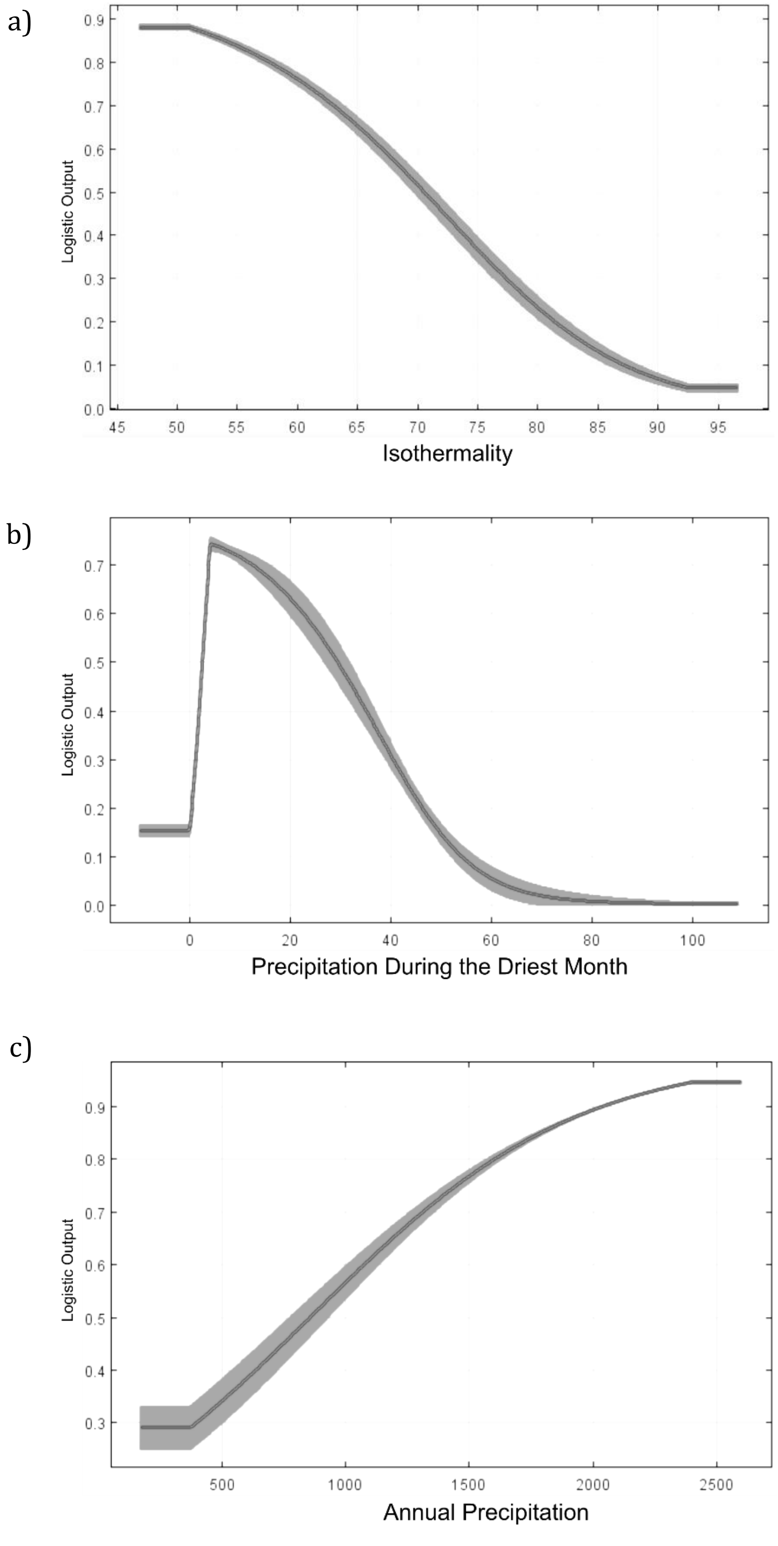


Figure 4.4. Response curves, by logistic output, in the ecological niche model for the top three contributing environmental variables: a) isothermality, b) precipitation during the driest month, and c) annual precipitation. The dark grey line shows the mean logistic output of the probability of presence for each environmental variable and the shaded grey area ± one standard deviation.

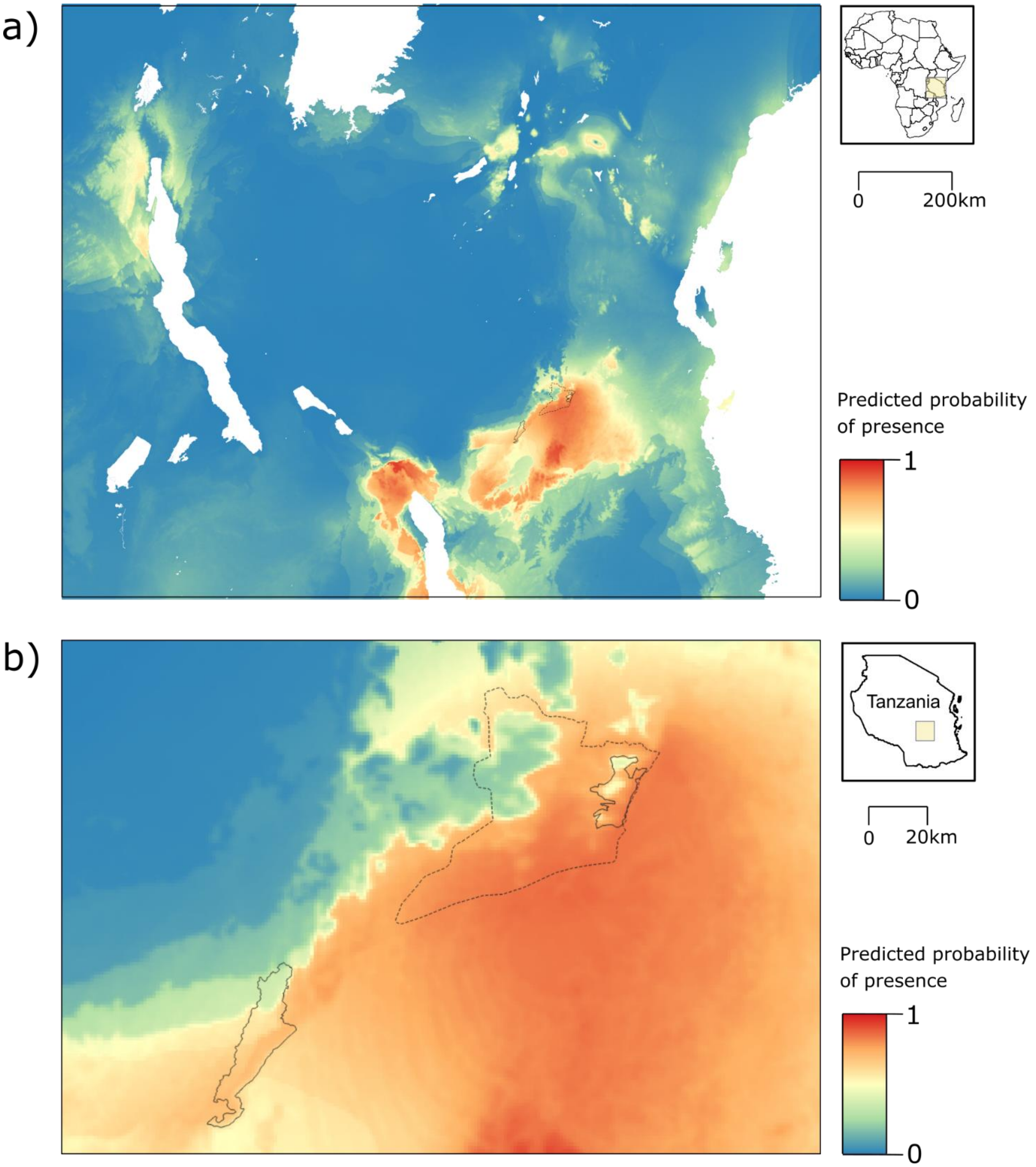


Figure 4.5. Estimated probability of habitat suitability for the Sanje mangabey (*Cercocebus sanjei*) within the Udzungwa Mountains based on near current (1960-1990) conditions across a) Tanzania and b) the Udzungwa Mountains.

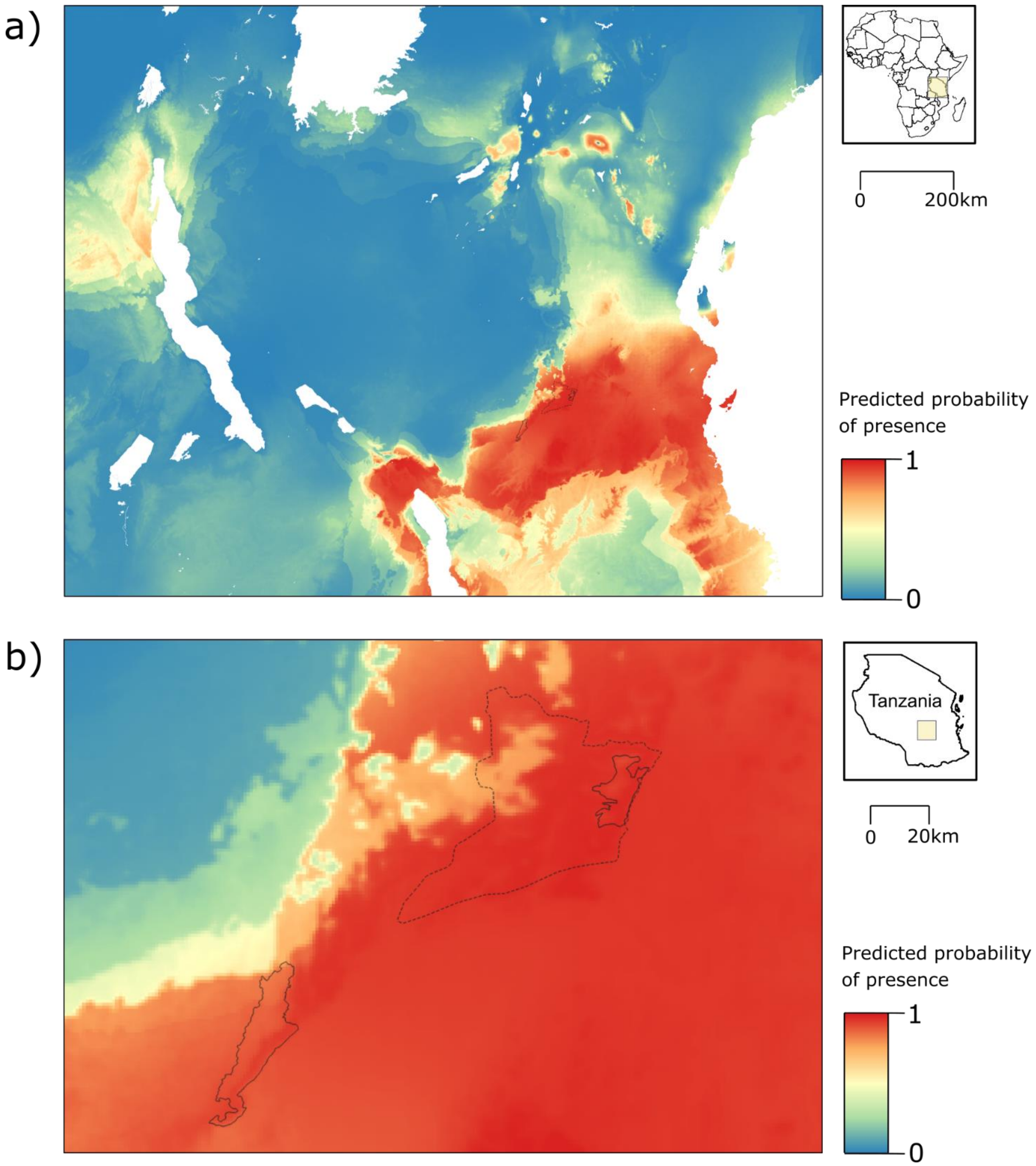


Figure 4.6. Estimated probability of habitat suitability for the Sanje mangabey (*Cercocebus sanjei*) within the Udzungwa Mountains during the Mid-Holocene (~6,000 YA) across a) Tanzania and b) the Udzungwa Mountains.

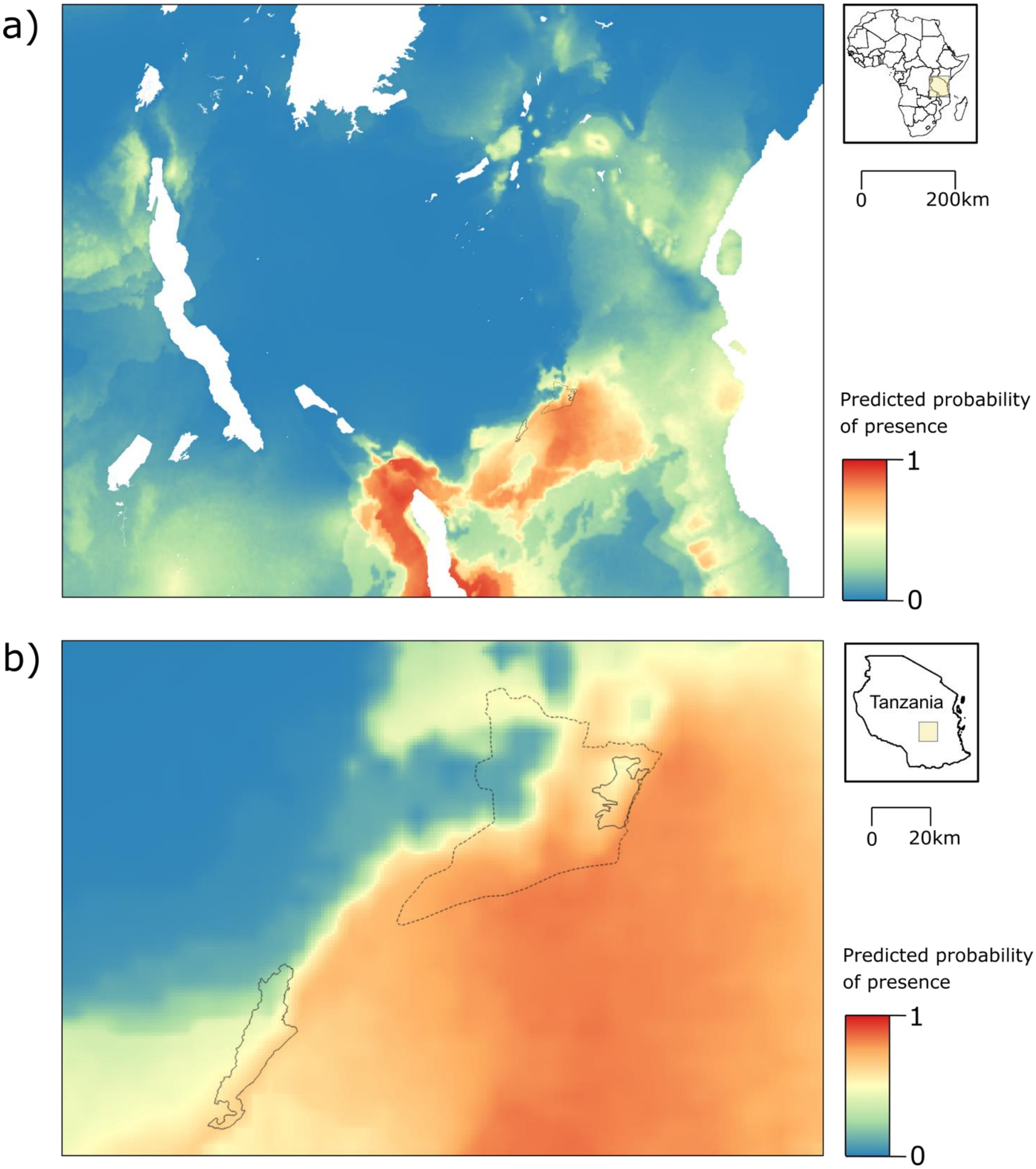


Figure 4.7. Estimated probability of habitat suitability for the Sanje mangabey (*Cercocebus sanjei*) within the Udzungwa Mountains during the Last Glacial Maximum (~22,000 YA) across a) Tanzania and b) the Udzungwa Mountains.

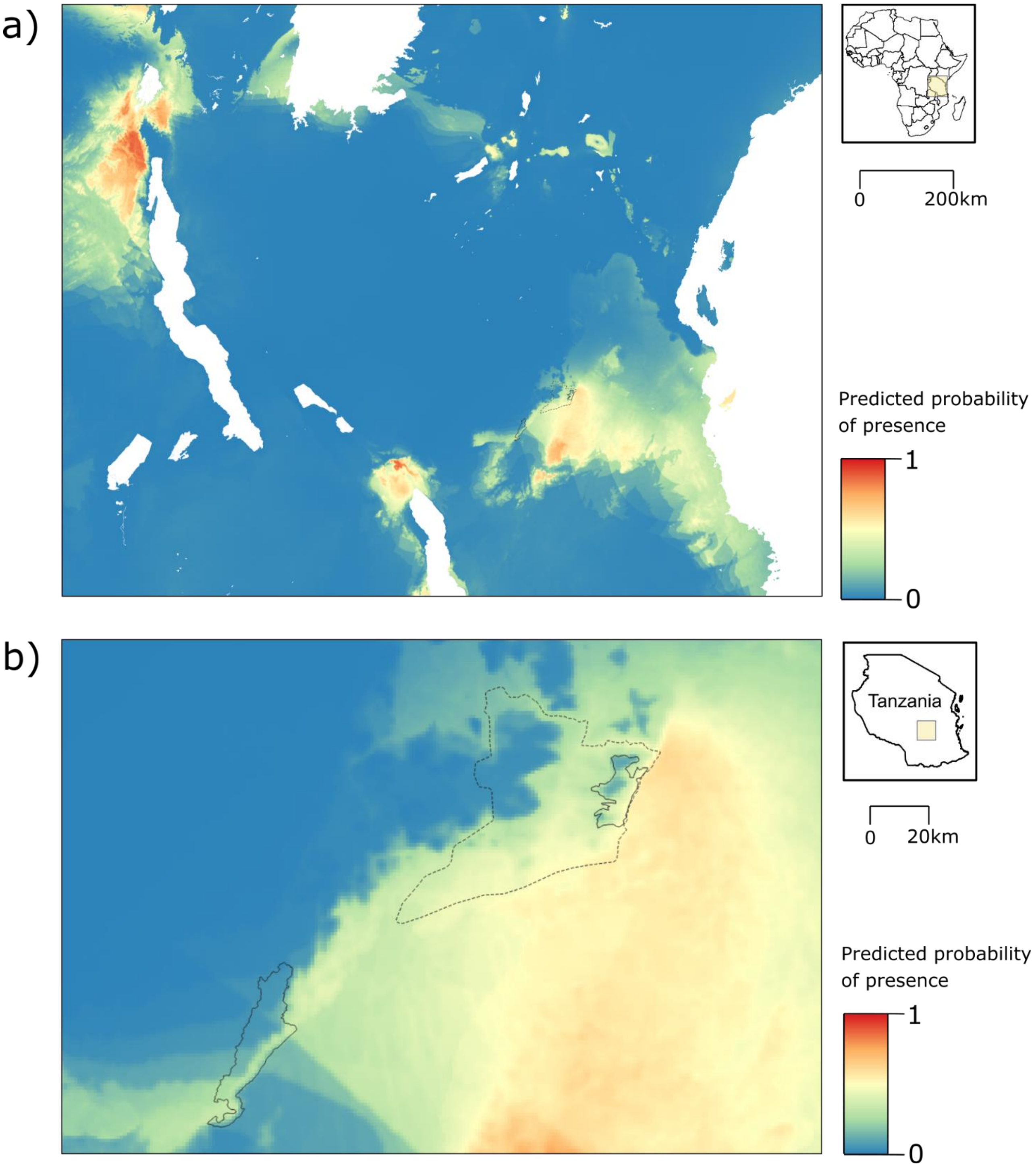


Figure 4.8. Estimated probability of habitat suitability for the Sanje mangabey (*Cercocebus sanjei*) within the Udzungwa Mountains during the Last Interglacial period (~120,000 – 140,000 YA) across a) Tanzania and b) the Udzungwa Mountains.

the Mwanihana and Uzungwa Scarp forest fragments. This information could be used to inform conservation action planning.

The two Sanje mangabey populations were found to be highly significantly differentiated, with no shared haplotypes. The most closely related haplotypes from each population had 16 mutational differences. The ecological niche modelling conducted for the Sanje mangabey over the past 140,000 years suggested suitable habitat expanded and contracted between the modelled time frames for the current, Mid-Holocene (~6,000 YA), Last Glacial Maximum (~22,000 YA) and Last Interglacial period (~120,000 – 140,000 YA) scenarios. Continuous suitable bioclimatic conditions appear to be present between the two forest fragments in which the extant Sanje mangabey populations are found. Therefore, the current isolation may have occurred on a more ancient timescale based on climatic conditions, however this most likely indicates that other factors (i.e., not climatic variables) may have contributed to the isolation of the populations.

4.4.1. Mitochondrial diversity and structure

Haplotype diversity in Mwanihana is likely representative of the diversity present in the population as the most frequent haplotype was sampled at all eleven independent sites, covering most of the distribution in that forest. In the Uzungwa Scarp, the number of haplotypes increased with increased sampling locations throughout the study. Further, the similar frequency of each of the four haplotypes present suggests that the diversity may increase with further sampling. For example, one haplotype (orange) was present in the far north and south of the Uzungwa Scarp and yet had 3 mutational differences to the next most closely related haplotype. This may be reflective of the diversity present but more likely that intermediates between these two haplotypes occur across the forest (Figure 4.2). Although the diversity in the Uzungwa Scarp may increase with further sampling, the differentiation between populations is likely to reflect the result in this study.

The mitochondrial haplotype and nucleotide diversity were significantly lower in Mwanihana (h = 0.056 ± 0.052; π = 0.00013 ± 0.00012) than in Uzungwa Scarp (h = 0.664 ± 0.055; π = 0.00417 ± 0.00041). It is unknown if this genetic pattern is common since there are no comparable studies for other *Cercocebus* species for population diversity for the mitochondrial control region. Compared to population genetic studies of other Papionin species, the Sanje mangabey Mwanihana population shows some of the lowest levels of nucleotide and haplotype diversity; however, the Uzungwa Scarp diversity was more reflective of the other studies (Table 4.6). In a study of *Theropithecus gelada*, for example, the lowest genetic diversity was attributed to a small population likely affected by genetic drift or a bottleneck (Zinner et al. 2018), although the haplotype network in that study did not reflect the star shape found for Mwanihana in this study.

Table 4.6. Comparison of nucleotide and haplotype diversity found in this study for the two populations of the Sanje mangabey (*Cercocebus sanjei*): Mwanihana (MW) and Uzungwa Scarp (US), and the two forests combined (ALL), with other Papionin species for fragments of the hypervariable (HV) regions of the mitochondrial control region. All species included show male-biased dispersal to be comparable with that of the Sanje mangabey.

Species		**Fragment**	**Nucleotide Diversity**	**Haplotype Diversity**	**Reference**
Cercocebus sanjei	MW	Partial HVI & HVII	0.00013 (0.00001 - 0.00025)	0.056 (0.004 - 0.108)	This study
	US		0.00417 (0.00376 - 0.00458)	0.664 (0.609 – 0.719)	
	ALL		0.022 (0.011 – 0.033)	0.642 (0.591 - 0.693)	
Theropithecus gelada		1800 bp mtDNA incl. HVI (462 bp)	0.003298 (0.00062 – 0.00732)	0.7332 (0.462 – 0.962)	(Zinner et al. 2018)
Papio ursinus griseipes		HVI (352 bp)	0.047533 (0.036 - 0.086)		(Burrell 2008)
Macaca assamensis		HVI (complete)	0.0118	0.962	(Khanal, Chalise, He, et al. 2018)
Macaca fascicularis		HVI (complete)	0.042 (0.00025 – 0.084)	0.73 (0.12 – 0.99)	(Badhan et al. 2015)

We did not expect to find a higher mitochondrial haplotype, nucleotide diversity and female effective population size in the Uzungwa Scarp than in Mwanihana, nor to find that the Uzungwa Scarp diversity was high and comparable to other species (Table 4.6). Mwanihana has historically had greater formal protection than the Uzungwa Scarp, having been protected by National Park regulations since 1992, and therefore it was expected that the population in Mwanihana would have experienced a lower decrease in effective population size and genetic diversity due to anthropogenic activities. The population size in each forest is relatively similar (Mwanihana: n = 1,712; Uzungwa Scarp: n = 1,455) with an inferred population size decrease in both populations over the last 20 years in both forests (Paddock et al. 2020); however this was not detected in our demographic analyses. This study found no evidence for population size change in the demographic analyses (Tajima's D, Fu's F_S, and BSP), bottlenecks or loss of diversity for each population, on a recent anthropogenic timescale, and demographic analyses suggest a stable female effective size for both. The levels of genetic diversity found in each population are inferred to have existed for an extended period of time, considering a more historic low level of genetic diversity. Therefore, this result is more likely related to a period of isolation on an ancient time scale of the current fragments of Uzungwa Scarp than to differences in formal protection between the two forests over the last few decades. Nevertheless, this result implies that the formal protection of Uzungwa Scarp should be improved since this result suggests most of the genetic diversity of the species is present in this forest.

Female Sanje mangabeys are known to be philopatric (McCabe and Emery Thompson 2013; Fernández 2017) and the significantly low number and diversity of mitochondrial haplotypes found in Mwanihana could represent a founder effect of a single female haplotype with the low diversity being maintained by the philopatric behaviour of females. Nuclear genome-wide evidence is required to corroborate this hypothesis. The star shape phylogeny, with the central haplotype likely ancestral to a descendant rare haplotype segregated by a single mutation, suggests the population may be expanding, with a mutation presenting new diversity to the population, or that an expansion occurred previously but other rare haplotypes were lost (Ferreri et al. 2011).

The two Sanje mangabey populations, separated by approximately 100 km, were found to differ significantly, with 95.49% of mitochondrial haplotype variation partitioned by population. This significant divergence resembles the differentiation pattern found for the sympatric Udzungwa red colobus in a genetic study using microsatellite markers (Ruiz-Lopez et al. 2016). In that study, the Uzungwa Scarp was found to be the most differentiated population with the highest proportion of private alleles compared to the other four forests in the study (Mwanihana, Ndundulu, Matundu, and Magombera; Figure 4.1), reflecting the difference in genetic diversity between populations in these forests in this study. The Uzungwa Scarp presently is in closer proximity to other forest blocks in the Udzungwa Mountains (Figure 4.1) and it is expected that primates inhabiting this forest patch would have had a greater connectivity and dispersal opportunities. However in the study by (Ruiz-Lopez et al. 2016), it was estimated that gene flow may have occurred through Matundu, Ndundulu and Mwanihana and Uzungwa Scarp and Magombera were found to be individual distinct units with restricted gene flow to these forests. The colobus populations inhabiting Uzungwa Scarp and Magombera forests were proposed by the authors to be representative of ancient lineages and that the current dispersal barriers associated to anthropogenic facilities (towns/villages shown in Figure 4.1) may be maintaining the pre-existing dispersal barriers and genetic distinctiveness.

Additionally, significant pairwise genetic differentiation in both microsatellite and mitochondrial DNA has been found previously between subpopulations of Harvey's duiker (*Cephalophus harveyi*) in the Uzungwa Scarp and Mwanihana, even with subpopulations present in intermediate forests throughout the Udzungwa Mountains (Bowkett et al. 2015). The differentiation was attributed to major rivers between forests acting as a barrier to dispersal. The major rivers highlighted in the study of Harvey's duiker as potential dispersal barriers (Bowkett et al. 2015) are likely to have also impacted the Sanje mangabey with the rivers Mngeta, Ruipa and Lumemo all positioned between Uzungwa Scarp and Mwanihana (Figure 4.1).

Low mitochondrial diversity combined with a low nuclear diversity could suggest a more historically low effective population size. For example, in the white-headed langur (*Trachypithecus leucocephalus*) both low mitochondrial and nuclear DNA diversity was found, suggesting a low effective size, likely due to a local founder event and a consequent ‘shallow’ evolutionary history, strongly influenced by recent declines due to anthropogenic activities (Wang et al. 2019). A population level study of genome-wide nuclear DNA diversity will help to further assess the phylogeographic history of the Sanje mangabey populations as use of only a few markers, or those with uniparental inheritance, can produce a biased result (Rakotoarivelo et al. 2019).

4.4.2. Ecological Niche Modelling

In the modelling of the current bioclimatic conditions and presence of Sanje mangabey, the most important environmental variables were a low range of isothermality (low levels of temperature variability from day to night and between seasons), moderate precipitation during the driest month and high annual precipitation (Figure 4.4). This is consistent with habitat requirements of stable forests which reflects the current distribution of the Sanje mangabey and the estimated historic dispersal patterns in line with forest habitat expansion and retraction. As the Sanje mangabey is considered flexible in its use of primary and secondary forest, and elephant-disturbed shrubland (McCabe et al. 2013), the populations may have successfully persisted in changing habitats over the recent past.

The ecological niche models for the Sanje mangabey estimate that suitable habitat has been present at the time points sampled; current (1960-1990; Figure 4.5), Mid Holocene (MH: ~6,000 YA; Figure 4.6), Last Glacial Maximum (LGM: ~22,000 YA; Figure 4.7), and the Last Inter-Glacial (LIG: ~120,000 – 140,000 YA; Figure 4.8), throughout the southern region of the Udzungwa Mountains. This region included the Uzungwa Scarp and Mwanihana where extant populations are found, and throughout the Kilombero Valley and the intermediate forest Matundu, suggesting bioclimatic conditions may be suitable to support dispersal. Given the evidence of genetic differentiation of the two populations of the Sanje mangabey provided by this work, this suggests that the structure of habitat and physical dispersal barriers may prevent dispersal rather than bioclimatic conditions, and that intermediate populations existed in other forest blocks within the region that have since become extinct, such as the suggestion by (Homewood and Rodgers 1981) of historic distribution throughout the Kilombero Valley lowland forests.

The results of the models reflect the stability of biodiversity within the forests of the Eastern Arc Mountains to global climatic changes (Fjeldsa and Lovett 1997; Marchant et al. 2007; Mumbi et al.

2008) and, combined with the estimated long-term stability in effective population size for the Sanje mangabey during this time, suggests a resilience in the species to climatic fluctuations. A general drying trend and aridification events in the region have been estimated since 1.86 MYA (Trauth et al. 2009). Studies of hominid evolution in East Africa showed the climate becoming dryer 1.6-1.8 MYA, coinciding with glacial cycles (with the last cycle 1 MYA) and with evidence of a shift from closed canopy forest to grassland (deMenocal 2004). The climate also fluctuated between relative extremes during glacial and interglacial cycles, ~41 KY cycles from 2.8 MYA to ~100 KY cycles after 1.0 MYA, with this overall aridification pattern broadly continuing to the present day (deMenocal 2004). The models here appear to reflect a level of fluctuation over the last 140,000 years, with estimated suitable conditions expanding and contracting between time points. Therefore, in the case of the Sanje mangabey, this may have resulted in a general trend of forest habitat contraction over, and prior to, the time period modelled in this study, resulting in fragmentation and isolation. Although not detected to be significant, the estimation of female effective population size by BSP analyses in the Uzungwa Scarp showed a slight increase since the Last Glacial Maximum (22,000 YA). This may be reflected in the niche models showing more suitable habitat since that time point, with a large increase during the Mid-Holocene, in the Scarp/south-west region allowing the population to expand in that time.

Analyses of a sediment core collected from a swamp near Iringa, located on the north-west boundary of the Udzungwa Mountains, estimated a shift in elevation of montane forests from 1700-1800 m 38 KYA, from 1850-1950 m 29-10 KYA and then to 2000 m 3.5 KYA to the present day (Mumbi et al. 2008). This could potentially have contributed to the isolation of suitable habitat, even if bioclimatic conditions were estimated to be suitable, creating physical barriers to dispersal with extant Sanje mangabey groups estimated to have greater occupancy with distance from the forest edge (Rovero et al. 2017). Over the last 3 MY, the widespread and sympatric *Cercopithecus* (*nictitans*) species are estimated to have been subject to the expansion and contraction of forests, although restricted to small and isolated refugia after 0.8 MYA (Butynski and Jong 2020), a similar time frame to the TMRCA between Sanje mangabey populations (0.77 MYA; Chapter Three). It is possible the gradual movement of montane forest to higher elevations (Mumbi et al. 2008), reducing habitat size to forest islands, resulted in the extinction of populations in isolated forest fragments of lower elevations. This may have led to further dispersal barriers when forests would fluctuate and expand, with intermediate populations no longer existing to disperse. Additionally, the contraction of suitable habitat may have led to greater competition for resources, such as the known competition between mangabeys and baboons, which may have been detrimental to Sanje mangabey. This competition may have also occurred with other primates in the region, including the Sykes' monkey and colobines, that live in association with the mangabey presently, and may have previously overlapped and competed with the kipunji.

Using a presence-only method, such as in this study, removes the need for absence data which requires intensive sampling, often unfeasible logistically and financially, to avoid unreliable absence data. However, similar limitations apply to using presence-only data where detectability of individuals may vary between sampling sites, due to landscape or habitat structure, and therefore occupied suitable habitat may not be recorded (Elith et al. 2011). Presence-only models are considered to perform well (Phillips and Dudík 2008), however future studies would benefit if presence-absence data and models were used, as they are less susceptible to sample selection bias and can provide information about prevalence (Elith et al. 2011). If this remains logistically difficult, increasing the sampling effort and locations across each habitat during future population surveys, as discussed by (Paddock et al. 2020), to increase the distribution and number of presence points across the sampling grids would benefit models.

4.4.3. Conservation implications

Previous studies that have identified and designated ESUs recommended that those units should be managed independently in conservation planning (e.g. Chaves et al. 2019; Mekonnen et al. 2018). A similar mtDNA phylogenetic structure and ancient divergence time was found between two populations of lion-tailed macaque (*Macaca silenus*), separated by the Palghat gap in Anamalai hills, southern India, and these two populations were designated as ESUs with conservation plans focussing on management of each ESU individually and maintaining and extending the habitat size for each (Ram et al. 2015). Protecting unique genetic diversity ensures the evolutionary potential of a species is maintained. Therefore, for the Sanje mangabey, both protected areas require separate protection, regeneration and potential expansion to allow the carrying capacity of each forest to increase and enable population sizes to expand.

As with the Udzungwa red colobus (Ruiz-Lopez et al. 2016), it is estimated differentiation and isolation has occurred on an ancient timescale, however the more immediate threats are the anthropogenic disturbances, such as human-set fires and the degradation of currently suitable habitat. Work in the Uzungwa Scarp to establish forest corridors between fragments has been ongoing, such as the Mngeta corridor connecting the Uzungwa Scarp to Matundu and the Kilombero Nature Reserve (Rovero and Jones 2012); however, these forests are at lower elevations and therefore may not be suitable for Sanje mangabey dispersal and/or occupation. Mangabeys exhibit avoidance behaviours from baboons and, as baboons are found in high densities at low elevations, this may have prevented mangabeys dispersing through the low elevation corridors. Alternatively, regenerating and extending both Uzungwa Scarp and Mwanihana forests at higher elevations would potentially be more beneficial. Immediate actions are needed to reduce habitat loss, fragmentation and poaching in the region, with monitoring and management of both populations required to reduce the risk of decline and loss of genetic diversity for the species. With active enforcement found to be successful in the National Park (Rovero et al. 2012; Rovero et al. 2015; Beaudrot et al. 2016; Oberosler et al. 2020), the Uzungwa Scarp Nature Reserve would benefit from a similar approach to ensure this evolutionarily distinct population is protected.

4.5. ACKNOWLEDGEMENTS

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CHAPTER FIVE

Viable or in a Vortex? Stochastic population modelling of the Endangered Sanje mangabey (*Cercocebus sanjei*)

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ABSTRACT

The Sanje mangabey (*Cercocebus sanjei*) is an Endangered primate, considered to be at greatest threat from loss of habitat from human encroachment and from poaching. Further, the species is divided into two small populations, each isolated in forest fragments situated 100km apart, and therefore under threat of loss of genetic diversity and increasing levels of inbreeding. Ecological Niche Models (ENM), and population modelling and simulations can potentially provide insight into the response of species to change in their environment, able to inform conservation planning by testing population responses to different management scenarios. This study aimed to develop and apply stochastic population modelling to assess the future viability of the Sanje mangabey populations without management intervention and the possible response of the two populations to four conservation approaches: i) reduced poaching, ii) increased carrying capacity, iii) reduced poaching and increased carrying capacity, and iv) reduced poaching, increased carrying capacity and the creation of a third population. An ENM was developed to estimate the climatic and biophysical parameters with the greatest importance for the presence of Sanje mangabey and was used to identify regions within the Udzungwa Mountains that could be suitable for the species to inform the conservation measures. The ENM used a Maximum Entropy model and current estimated bioclimatic and biophysical variables for the Udzungwa Mountains. Presence locations (n=18) from a previous ENM of historic distribution for the species were used, distributed across most of the estimated extent of the two populations. The population viability modelling was conducted using the well-established software VORTEX, and simulations used existing life history data for the Sanje mangabey and data from closely related species where knowledge gaps existed. The ENM estimated Sanje mangabey presence to be highest at low compound topographic index values, indicating a preference for regions within small catchment areas and steep slopes, which is consistent with its known distribution in montane forests. The PHVA estimated that the current scenario without intervention for each population would result in declining population sizes, deterministically tracking the estimated decreasing carrying capacity in each forest. The most successful conservation management scenario approach modelled was a combination of increasing carrying capacity in both forests and the elimination of the impact of poaching for the Uzungwa Scarp population. Compared to the no intervention scenario, the number of extant individuals, stochastic growth rate, and retained heterozygosity were higher, and inbreeding decreased. We concluded that two priority conservation actions could be recommended: i) increase protection of the Uzungwa Scarp to reduce the loss of carrying capacity and decrease poaching activities, and ii) increase carrying capacity through forest regeneration in the montane regions within each forest block that were estimated to show high presence probability for the Sanje mangabey in the ENM study. These actions must be taken in conjunction with local communities to increase awareness and to develop alternative livelihoods from patrolling and monitoring the forests.

5.1. INTRODUCTION

The Sanje mangabey (*Cercocebus sanjei*) is an endemic primate present in two forest blocks within the Udzungwa Mountains, south-central Tanzania, and is considered Endangered by the International Union for Conservation of Nature (IUCN) due to its small population size, limited distribution and fragmentation of the two known populations, and threats from poaching (McCabe *et al.* 2019; Figure 5.1). Whilst the Udzungwa Mountains forests are estimated to be a combined total of 1600 km^2 (Marshall *et al.* 2010), the Sanje mangabey is restricted to an estimated total of 464.5 km^2 in just two forest fragments: Mwanihana, in the Udzungwa Mountains National Park (150 km^2), and the Uzungwa Scarp Nature Reserve (314.5 km^2; Figure 5.2). Although similar in elevation, habitat composition, mean annual rainfall and mean temperature range (Hegerl *et al.* 2017), the forests differ primarily in the threat of poaching, being significantly higher in the lesser protected Uzungwa Scarp (Oberosler *et al.* 2019). As a result, a study comparing the biodiversity of the two forests found a lower species richness, an altered trophic structure and lower occurrence probabilities for most species surveyed in the Uzungwa Scarp (Oberosler *et al.* 2019; Oberosler *et al.* 2020).

Figure 5.1. A snare found and removed in the northern region of Mwanihana forest, Udzungwa Mountains National Park, during a population survey of the Sanje mangabey (Paddock *et al.* 2020). There was clear, fresh disturbance of the ground around the tree, suggesting a large mammal had been trapped but managed to escape. Photo credit: C. L. Paddock.

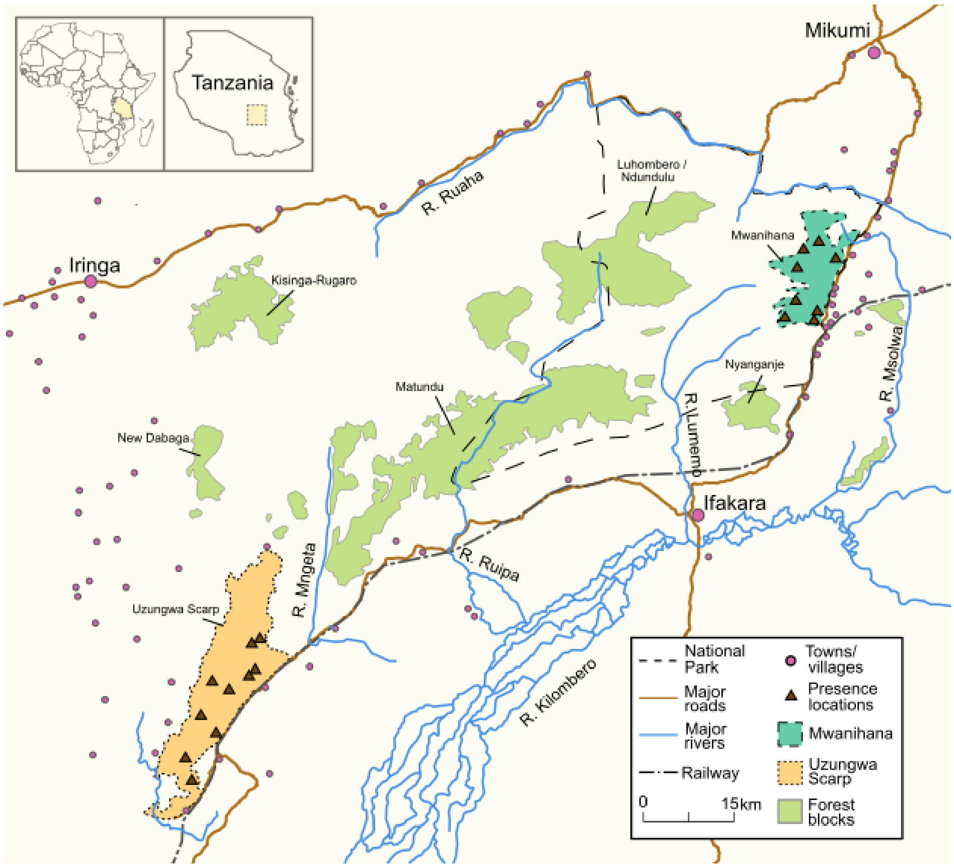

Figure 5.2. A map of the Uzungwa Mountains, Tanzania, with the forests in which the Sanje mangabey (*Cercocebus sanjei*) populations are found: Mwanihana (north-east; green) and Uzungwa Scarp (south-west; yellow). The presence locations used in the Ecological Niche Model in this study are shown (brown triangles).

The Uzungwa Mountains National Park was established in 1992, however other forests in the region were protected at most with Forest or Nature Reserve status. These forests did not receive effective protection and encroachment has continued (Rovero *et al.* 2012). In their study, Hegerl *et al.* (2017) noted a marked difference in budget and staffing between the Uzungwa Scarp and Mwanihana ($1,000 compared to $400,000 USD, and 1 compared to 78 permanent staff members, respectively). The Uzungwa Scarp was upgraded to Nature Reserve status from Forest Reserve in 2017, providing stricter regulations but still requiring greater active law enforcement. Hunting in the Uzungwa Scarp was reported to be at its most prevalent from 1965 to 1975, according to interviews with local people, a time period which coincided with the extinction of large mammals, such as elephants (*Loxodonta Africana*), buffalo (*Syncerus caffer*) and leopard (*Pathera pardus*; Rovero *et al.* 2012). Hunting continued but switched in the mid-1990s from commercial hunting of profitable large mammals, to subsistence hunting of smaller mammals, using methods such as snares and dogs that represented a threat to all terrestrial and semi-terrestrial mammals in the region, including the Sanje mangabey (Rovero *et al.* 2012). In addition to hunting, the Uzungwa Scarp is more affected than Mwanihana forest by habitat destruction and disturbance (e.g. natural resource extraction; Rovero *et al.* 2012). Human disturbance; including snares, fires, gunshots, and deforestation, was found to increase by 19% between 1998 and 2008 (Rovero *et al.* 2010). Villages exist only on the eastern boundary of Mwanihana forest, with all other boundaries surrounded by other protected regions, however the Uzungwa Scarp is surrounded by villages, with no protected regions to act as a buffer for access to the reserve (Oberosler *et al.* 2019; Figure 5.2).

The life history of the Sanje mangabey raises concerns about the viability of the species as a large-bodied, K-selected species (species that have few offspring and invest significantly in parental care). The Sanje mangabey is vulnerable to population declines with its relatively large body size and the link of this trait to high extinction risk in mammals (Cardillo *et al.* 2005). As a species with a relatively small population size, especially when considering the isolation of each population and individually small population size (Paddock *et al.* 2020), the Sanje mangabey is at greater risk of local extinctions due to stochastic processes. Their large home range and primarily frugivorous diet could also present a higher risk of extinction, as found in a comparative analysis of 93 primate species and their conservation status (Lootvoet *et al.* 2015). This isolation increases the chance of inbreeding, reduced effective population size, loss of genetic diversity, genetic drift, and the loss of adaptive potential within each forest.

5.1.1. Modelling Viability and Conservation Approaches

Stochastic population models utilise the intrinsic life history parameters of a species alongside the extrinsic characteristics of its habitat and environment, including the probability of stochastic catastrophic events (e.g. disease and extreme weather; Lacy *et al.* 2018), to predict the response and viability of populations (using parameters such as probability of extinction, stochastic growth rates, and levels of genetic diversity). The models can also be developed to predict the viability of the population in response to estimated changes in its environment, such as increased human disturbance (e.g., poaching) and climatic change (e.g., reduced carrying capacity of habitats with shifting locations of suitable conditions). Likewise, the impact of conservation actions can be modelled to estimate the response of populations to different management approaches such as forest regeneration, forest corridors, and translocation of individuals. Stochastic modelling of the Tana River mangabey (*Cercocebus galeritus*), considered to be one of the most closely related *Cercocebus* species to the Sanje mangabey (Chapter Three), found population numbers required to achieve the desired effective population size (based on $N_e = 500$) would be unrealistic in the limited available habitat (not considering isolation: N = 3,600; considering isolation of river banks: N = 9,000; Kinnaird and Brien 1991). At the time of the analysis, the Tana River mangabey population size put it at significant risk of extinction over the following 50-100 years and would require intervention to reduce the risk, suggesting the creation of habitat corridors to encourage gene flow between population and improving the quality of existing degraded habitats. Translocations were not recommended due to logistical issues and unknown social consequences (e.g. male takeover dynamics and the risk of infanticide; Kinnaird and Brien 1991). However, it is worth noting here that forest corridors and measures to encourage gene flow between Sanje mangabey populations would not be considered with the two populations representative of ESUs (Chapter Three; Chapter Four).

Additional information on suitable habitat for the species can be estimated through ecological niche modelling (ENM). ENM can provide an insight into the importance of bioclimatic and biophysical traits in the region in which the species are found, that correlate to produce a scale of predicted probability of presence of that species. ENMs can highlight priority and other viable habitat for concentrated conservation efforts, and this information can then be incorporated into viability assessments to assess management options. For example, Bonfim *et al.* (2019) used this approach to inform conservation planning for the red-billed curassow (*Crax blumenbachii*), an Endangered species in the northern Atlantic Forest, by identifying suitable priority habitat areas with an ENM that would allow for long term viable populations for the species. Using stochastic models, the authors elaborated recommendations to: i) increase protection in regions with extant populations, ii) active reintroduction and protection of regions where the curassow existed previously, and iii) increase connectivity between fragments to allow dispersal and gene flow (Bonfim *et al.* 2019).

5.1.2. Aims and Hypotheses

This study aimed to use the current distribution of the Sanje mangabey and both bioclimatic and biophysical variables in an ENM. The results of this model were to be used to identify the variables that have the greatest contribution towards mangabey presence. Additionally, extrapolating the estimated suitable habitat conditions across the Udzungwa Mountains, the results would be used to identify other regions that may provide similar conditions. These regions can then be assessed as to whether an extension of the current forests or forest corridors may be a viable option. Secondly, this study aimed to model the current and projected population viability of the Sanje mangabey by conducting stochastic population modelling with the Vortex simulation and modelling software. The estimated viability, measured using parameters such as number of extant individuals, inbreeding occurrence, heterozygosity, and stochastic growth rate, were used to assess the extinction risk of the species. Finally, conservation strategies were modelled to investigate approaches that are most successful in minimising the loss of viability, and recommendations are made.

5.2. MATERIALS AND METHODS

The Sanje mangabey is divided into two populations: Mwanihana (7°40′–7°57′S, 36°46′–36°56′E) and Uzungwa Scarp (8°14′–8°32′S, 35°51′–36°02′E; Figure 5.2). Mwanihana forest is protected by National Park regulations, situated inside the Udzungwa Mountains National Park boundary. Uzungwa Scarp is a Nature Reserve with regulations and protection however this is to a lesser degree than the National Park, and active protection is less frequent.

5.2.1. Ecological Niche Modelling

An ENM maximum entropy framework was used to calculate the probable distribution of the Sanje mangabey across the Udzungwa Mountains region using the software Maxent v 3.4.1 (Phillips *et al.* 2006). Geographic coordinates for presence locations (n=18) of the Sanje mangabey prepared for a previous ENM investigating historical habitat suitability were used in this study (Chapter Four; presence locations shown in Figure 5.2). In this study, both biophysical and bioclimatic variables were prepared to estimate areas of probable occurrence for current times (1970-2000; all variables listed in Table 5.1).

The 19 bioclimatic variables for the current scenario (1970-2000; 30 sec) were downloaded from WorldClim 2 (https://www.worldclim.org/). Elevation data were extracted from Shuttle Radar Topography Mission (SRTM) using R (R Core Team 2018). Biophysical data: aspect, integrated moisture index, heat load index, curvature, compound topographic index, surface relief ratio, roughness, surface exposure index, and slope position index, were extracted from ASTER Global Digital Elevation Model layers (ASTER GDEM: NASA/METI/AIST/Japan Spacesystems, and U.S./Japan ASTER Science Team, 2019) using the Geomorphometry and Gradient Toolbox (Evans, Oakleaf and Cushman, 2014) in ArcMap 10.5.1. Landsat 8 satellite images (filtered for <10% cloud cover) were downloaded from USGS EarthExplorer (https://earthexplorer.usgs.gov/) to extract brightness, wetness and greenness using a tasselled cap transformation of values extracted from the images using the '*RStoolbox*' package in R. Normalised Difference Vegetation Index (NDVI; vegetation cover) values were calculated using the bands of the Landsat satellite images as a ratio of the visible red (band 4) and near infrared (band 5) bands.

Layers were cropped to the same extent, creating a study area within the boundaries of 34.75-37.25°E, 9.25-7.25°S, encompassing the Udzungwa Mountains region in south-central Tanzania where the Sanje mangabey is found. Layers were projected to the same co-ordinate reference system (WGS84:4326) and resampled to 30s to reflect the resolution of the bioclimatic layers using the *raster* package in R (R Core Team 2018). A Pearson's correlation (r) test was used in R (R Core Team 2018) to measure the correlation between all variables (Table 5.1). Where variables were highly correlated ($r > 0.7$), one variable was retained prioritising the broader variable (e.g.. annual mean temperature over specific seasonal variables). This resulted in 21 of the 31 layers being removed from the analyses (Table 5.1).

To estimate the best fitting model, the ENMeval package (Muscarella *et al.* 2018) in R (R Core Team 2018) was used to model combinations of the regularisation multiplier and feature classes (linear: L, quadratic: Q, product: P, hinge: H, and threshold: T). Ten regularisation multiplier values (1-10) and the combinations of five feature classes: 'L', 'LQ', 'H', 'LQH', 'LQHP' and 'LQHPT' were used. The best model is estimated where ΔAIC = 0. The model's Area Under the Receiver Operating Characteristic (AUC) curve was estimated in Maxent as a measure of the model performance. An AUC value of 0.5 for a model indicates random prediction and values closer to 1 indicate better model performance. The AUC in a presence-only model indicates whether the probability values of the model estimated for presence points are higher than those for the values of the background points. The model, with the features and regularisation multiplier determined to be most appropriate, was run using the cross-validation replicate run type for 25 replicates, and with a maximum of 10,000 background points (discussed in Chapter Four). The Maxent parameters were set to logistic output and 5,000 iterations for each simulation.

Response curves were generated for the current model to estimate the contribution of each predictor variable on the probability of presence of the Sanje mangabey. Jack-knife of regularised training gain was estimated for each predictor variable to estimate the contribution of each parameter to presence singularly, when acting as the only variable and when individually removed from the full model. The final model was converted into a minimum training presence (MTP) binary model. The MTP threshold uses the lowest suitability value in which the species is known to be present from the model to set the minimum threshold level for the estimated distribution. Regions below the MTP threshold would then be excluded from the binary MTP output, considered as absence.

Table 5.1. Biophysical and bioclimatic variables considered for an ecological niche model (ENM) for the Sanje mangabey in the Udzungwa Mountains, Tanzania. Non-correlated variables included in the model are indicated by a tick in the final column. Variables that were found to be highly correlated (Pearson's correlation index r > 0.7) were not included in the Maximum Entropy Species Distribution Model.

Variable	Description	Source	
Biophysical			
Elevation	Metres above sea level	SRTM	
Compound topographic index	Steady state wetness index; (Gessler *et al.* 1995; Moore *et al.* 1993)	ASTER GDEM	✓
Curvature	Concave/convex surface index; (McNab 1993; Bolstad and Lillesand 1992; McNab 1989)		

Heat load index	Direction of slope and amount of solar radiation received; (McCune and Keon 2002)		
Integrated moisture index	Estimate of soil moisture; (Iverson *et al.* 1997)		✓
Roughness	Surface roughness; (Riley *et al.* 1999)		
Site exposure index	Aspect is rescaled to north/south and then weighted by slope steepness; (Balice *et al.* 2000)		✓
Slope position index	Average neighbour slope value subtracted from the focal position; (De Reu *et al.* 2013; Guisan *et al.* 1999)		
Surface relief	Rugosity in surface; (Pike and Wilson 1971)		✓
Brightness (TC)	Soil cover	Landsat 8	✓
Greenness (TC)	Vegetation cover		✓
Wetness (TC)	Soil and canopy moisture		
NDVI	Photosynthesising vegetation cover		
Bioclimatic			
Bio1	Annual mean temperature	WorldClim 2	✓
Bio2	Mean diurnal range: (Mean max. temp – min. temp)		
Bio3	Isothermality: (Bio2/Bio7) *100		
Bio4	Temperature seasonality: (Standard Deviation * 100)		✓
Bio5	Max. temperature warmest month		
Bio6	Min. temperature coldest month		
Bio7	Temperature annual range: (Bio5 – Bio6)		
Bio8	Mean temperature of wettest quarter		
Bio9	Mean temperature of driest quarter		
Bio10	Mean temperature of warmest quarter		
Bio11	Mean temperature of coldest quarter		
Bio12	Annual precipitation		
Bio13	Precipitation of wettest month		
Bio14	Precipitation of driest month		
Bio15	Precipitation seasonality		✓
Bio16	Precipitation of wettest quarter		
Bio17	Precipitation of driest quarter		
Bio18	Precipitation of warmest quarter		✓
Bio19	Precipitation of coldest quarter		

5.2.2. Stochastic Population Modelling

Stochastic modelling was conducted using life history data previously collected in studies of a habituated Sanje mangabey group in Mwanihana or from closely related species where information was not available. Modelling and simulations were conducted in VORTEX v.10 (Lacy *et al.* 2018). Each model was run for 1000 iterations for a time span of 100 years and extinction was defined by only one sex remaining.

The baseline scenario was developed by incorporating known baseline parameters and by sensitivity testing of unknown parameters for the species (Table 5.2). Dispersal was not modelled in the baseline scenario due to the geographic isolation of the two populations and their long-term genetic isolation (estimated to be of 0.77 MY; Chapter Three), which means they are genetically distinct and likely separate evolutionarily significant units (Chapter Three; Chapter Four). The reproductive system variables (Table 5.2) were informed by studies on the closely related Tana River mangabey (*Cercocebus galeritus*; Wieczkowski and Butynski 2013) and infant mortality rates from the study of the habituated group in Mwanihana (McCabe and Fernández 2018; Table 5.2). Further mortality estimates used were informed by the life tables for the blue monkey (*Cercopithecus mitis*; Bronikowski *et al.* 2016) as a medium-sized Cercopithecine species with a similar age to adulthood and first dispersal, average lifespan, and the same dispersing sex, and therefore expected to show relatively similar intrinsic mortality rates to the Sanje mangabey from the data available for other species (Table 5.2). The data required to estimate Sanje mangabey life history tables were not available for a more closely related species for intervals over the lifespan of the study species. The use of blue monkey life history tables does present a limitation due to the differences in social structure with the Sanje mangabey, therefore results using these values should be treated with caution. The rates were averaged and adjusted to fit into the VORTEX model age and sex classes. The percentage of females breeding each year was estimated at 63%, as estimated for *Cercocebus galeritus* (Wieczkowski and Butynski 2013). Density dependent reproduction parameters were modelled over a range of values to estimate the impact on the growth rate of the population compared to the baseline 63% females breeding each year:

$$P(0) - ((P(0) - K) \times \left(\left(\frac{N}{K}\right)^{2}\right) \times (\frac{N}{0 + N})$$

Mortality Parameter Sensitivity Testing

Mortality estimates were tested for sensitivity for the effect of increased (+10% and +20% of the baseline value) or decreased mortality (-10% and -20% of the baseline value) for each age/sex class on the stochastic growth rate (r). The significance of the association between mortality of each age/sex class and the population growth rate was measured using a linear regression in R (R Core Team 2018).

Table 5.2. Baseline life history parameters for the VORTEX population and habitat viability model for the Sanje mangabey (*Cercocebus sanjei*).

Parameter	Value	Justification	Citation
Species Description			
Inbreeding depression	N/A	Inbreeding was not modelled due to the two populations being representative of ancient lineages and show a stable effective population size	*Chapter Three; Chapter Four*
EV correlation between reproduction and survival	0.2		
EV correlation among populations	1		
Reproductive System			
System	Polygynous	No data for the reproductive system of the Sanje mangabey is currently available, therefore these data are based on the closely related Tana River mangabey (*Cercocebus galeritus*)	*Wieczkowski and Butynski (2013); Kinnaird and Brien (1991)*
Age of first offspring females	6		
Max. age of female reproduction	19		
Age of first offspring males	7		
Max. age of male reproduction	19		
Max. lifespan	19		
Max. number of broods per year	1		
Max. number of progeny per brood	1		
Sex ratio at birth – in % males	50		
Make offspring dependent on their dam for *x* years	1		
Density dependent reproduction	Y; % P(0) = 63; % K = 50; A = 0; B = 2		

Reproductive Rates			
% adult females breeding	*VORTEX function*	These data were estimated from preliminary observational data collected from the habituated group study	*G. McCabe, unpublished data*
SD in % breeding due to EV	20%		
Distribution of broods per year	0 broods: 0% 1 brood: 100%		
Specify the distribution of number of offspring per female per brood	Specify exact distribution; 1 Offspring: 100%		
Mortality Rates % (SD due to EV)			
0 to 1	39.8 (±4.1)	From habituated group study	*McCabe and Fernández (2018)*
1 to 2	5 (±1.0)	No long-term mortality rates for age classes after infants are available for the Sanje mangabey. These data used here are modified from life history tables for *Cercopithecus mitis*	*Bronikowski et al. (2016)*
2 to 3	2 (±0.4)		
3 to 4	2 (±0.4)		
4 to 5	2 (±0.4)		
5 to 6	2 (±0.4)		
After age 6 (females)	3 (±0.6)		
6 to 7 (males)	2 (±0.4)		
After age 7 (males)	3 (±0.6)		
Mate Monopolisation			
% males in breeding pool	100		

Estimated Current Scenario with No Extinction Pressures

The estimated current scenario with no extinction pressures was developed using the values in Table 5.2, the estimated population sizes from Paddock *et al.* (2020), and a carrying capacity for each forest fragment (Table 5.3). Carrying capacity for each forest was estimated using the following calculation:

$$K = (habitat\ size\ \div home\ range\ size) \times group\ size$$

Group size was taken from Paddock, Bruford, & McCabe (2020; Mwanihana: 39.2, Uzungwa Scarp: 31.7), habitat size from (Marshall et al., 2010; Mwanihana: 150.59 km^2, Uzungwa Scarp: 314.48 km^2) and home range size estimate from (Ehardt, Jones, & Butynski, 2005; 2 km^2).

Table 5.3. Estimated population size and carrying capacity for the Sanje mangabey (*Cercocebus sanjei*)

Initial population size		
Initial population size	Mwanihana: 1,712 Uzungwa Scarp: 1,455	(Paddock *et al.* 2020)
Carrying capacity		
Carrying capacity (K)	Mwanihana: 1,968 Uzungwa Scarp: 3,323	(Marshall *et al.* 2010; Paddock *et al.* 2020; Ehardt *et al.* 2005)

Extinction Pressure Sensitivity Testing

The impact of varying levels, high to low, of anthropogenic extinction pressures were measured on the current populations of Sanje mangabey for poaching (mortality: baseline, +1%, +2%, +3%, +4% mortality for adult males and females) and deforestation (carrying capacity: baseline, -0.1%/year, -0.5%/year and -1%/year; Table 5.4). The output was compared to the estimated current scenario with no extinction pressures using pairwise comparisons to test if the final extant population size, probability of extinction, heterozygosity, and number of alleles had been significantly influenced by the pressure parameters set. This was conducted using the vortexR package (Pacioni and Mayer 2017) in R Version 3.5.2 (R Core Team 2018) and significance was measured when $p < 0.05$.

An estimated current model scenario was developed with varying combinations of extinction pressure estimates (Table 5.5). These were estimated to be representative of the potential current pressures for the Sanje mangabey. Scenarios were only run if the threat levels were higher in the Uzungwa Scarp than Mwanihana to reflect the greater protection of the forest under National Park regulations. The disease parameter was set to a conservative fixed value, independent of the population, as the impact is currently unknown. It is unknown whether the species is affected by inbreeding therefore the scenarios were modelled conservatively without inbreeding depression pending further investigation.

Table 5.4. Extinction pressures modelled for each Sanje mangabey population. Variation in values were modelled for mortality and carrying capacity pressures individually to estimate the impact of each threat on the populations in isolation. Baseline parameters from the current scenario without extinction pressures are shown in bold.

	Mwanihana	**Uzungwa Scarp**
Mortality Rates: Poaching		
After age 6 (females) & after age 7 (males)	**3 (±0.6)** / 4 (±0.8) / 5 (±1) / 6 (±1.2)	**3 (±0.6)** / 4 (±0.8) / 5 (±1) / 6 (±1.2)
Carrying capacity		
Future change in K?	Y	Y
Over how many years?	100	100
% Annual increase or decrease	**0** / -0.1 / -0.5 / -1	**0** / -0.1 / -0.5 / -1

Estimated Current Scenario with No Intervention

The model was run using the values for extinction pressures considered to reflect the present day scenario for the populations in Mwanihana and Uzungwa Scarp, combining parameters in Table 5.2, Table 5.3, and Table 5.5. The model was run for 100 years for 1000 iterations to estimate the population viability with no conservation actions.

Table 5.5. Extinction pressures modelled for each Sanje mangabey population, based on the results of models from Table 5.4, estimated to be reflective of the current threat levels to the species.

	Mwanihana	**Uzungwa Scarp**
Mortality Rates: Poaching		
After age 6 (females) & after age 7 (males)	3 (±0.6)	4 (±0.8)
Disease		
Local	Y	Y
Frequency (%)	50	50
Severity: Reproduction	1	1
Severity: Survival	0.99	0.99
Carrying capacity		
Future change in K?	Y	Y
Over how many years?	100	100
% Annual increase or decrease	-0.1	-0.5

Conservation Management Scenarios

The conservation management scenarios tested were: i) increased carrying capacity; ii) reduced poaching, iii) increased carrying capacity and decreased poaching; and iv) increased carrying capacity, decreased poaching, and initiating and building a new population.

Increased carrying capacity was modelled at a conservative level, considering a 0.005% increase in habitat each year for each population. This percentage was used as it may be manageable logistically, equating to 0.75 km^2 in the first year for Mwanihana and 1.5 km^2 in Uzungwa Scarp. Estimating the impact of minimal increases in carrying capacity is used to show the potential recovery even at a low levels of action. Reduced poaching was measured as removing the increased mortality for adults in the Uzungwa Scarp population, reflecting the same mortality rates as the highly protected Mwanihana population. In the third conservation scenario, the above carrying capacity and reduced poaching mortality were combined together, assuming increased protection of the populations and ranger activity would result in both measures.

Translocations between the two populations were not considered due to the high genetic divergence found previously (Chapter Three; Chapter Four) between the two populations remaining investigations required into the potential presence or absence of adaptive differentiation and the unknown potential detrimental impact on survival of translocated individuals. However, although also likely logistically and ethically unrealistic due to the limited biological understanding of the species, the fourth conservation scenario modelled increased K and elimination of poaching, and the impact of moving a small group of 15 individuals to a forest identified as potentially suitable in the ENM model (see results). Luhombero (~20 km west of Mwanihana forest; Figure 5.2) was chosen as a theoretical example forest to model the creation of a new population (see ENM results), which is estimated to be 230 km^2 and modelled using a carrying capacity of 3,645 individuals. If a measure to create a new population is deemed appropriate in the future, this model provides an insight to how the populations may respond demographically. Practical limitations of this approach are considered and described in the Discussion. Only one existing population would supplement the new population due to the two extant populations being ESUs. Therefore the Uzungwa Scarp is used as an example here, as the more threatened population and ESU requiring greater protection. This new population was modelled to be supplemented with a further four female and one male adult from Uzungwa Scarp every 20 years.

5.3. RESULTS

5.3.1. Ecological Niche Modelling

The best fitting model was estimated to include a linear feature class and a regularisation multiplier of 1 (AICc = 364.02, ΔAIC = 0; Figure 5.3). The model was run for 25 replicates and the average test AUC was 0.907 ±0.088. The top three contributing environmental variables were compound topographic index (74.4%), bio15 (11.4%), and brightness (6.9%). The compound topographic index variable had the greatest permutation importance (76%; Table 5.6).

Table 5.6. Percent contribution and permutation importance for each variable in the Ecological Niche Modelling for the Sanje mangabey (*Cercocebus sanjei*).

Variable	Percent contribution	Permutation importance
Compound topographic index	74.4	76
Precipitation seasonality (Bio15)	11.4	9.8
Brightness	6.9	7.5
Integrated moisture index	4.4	4.5
Temperature seasonality (Bio4)	1.6	0.4
Surface exposure index	1	1.1
Precipitation of the warmest quarter (Bio18)	0.3	0.6
Surface relief	0	0.1
Greenness	0	0
Annual mean temperature (Bio1)	0	0

The ENM estimated moderately suitable habitat (>0.3 predicted probability, above the MTP threshold) in other forest fragments within the Udzungwa Mountains, including New Dabaga north of the Uzungwa Scarp and Luhombero within the UMNP to the west of Mwanihana (forest locations: Figure 5.2; ENM: Figure 5.3). The intermediate forest Matundu, situated between Mwanihana and Uzungwa Scarp, was estimated to have potentially suitable habitat at the far west and east regions of the forest, however the central area showed lower predicted presence probability and may represent a potential dispersal barrier if mangabeys were introduced to that forest (Figure 5.3).

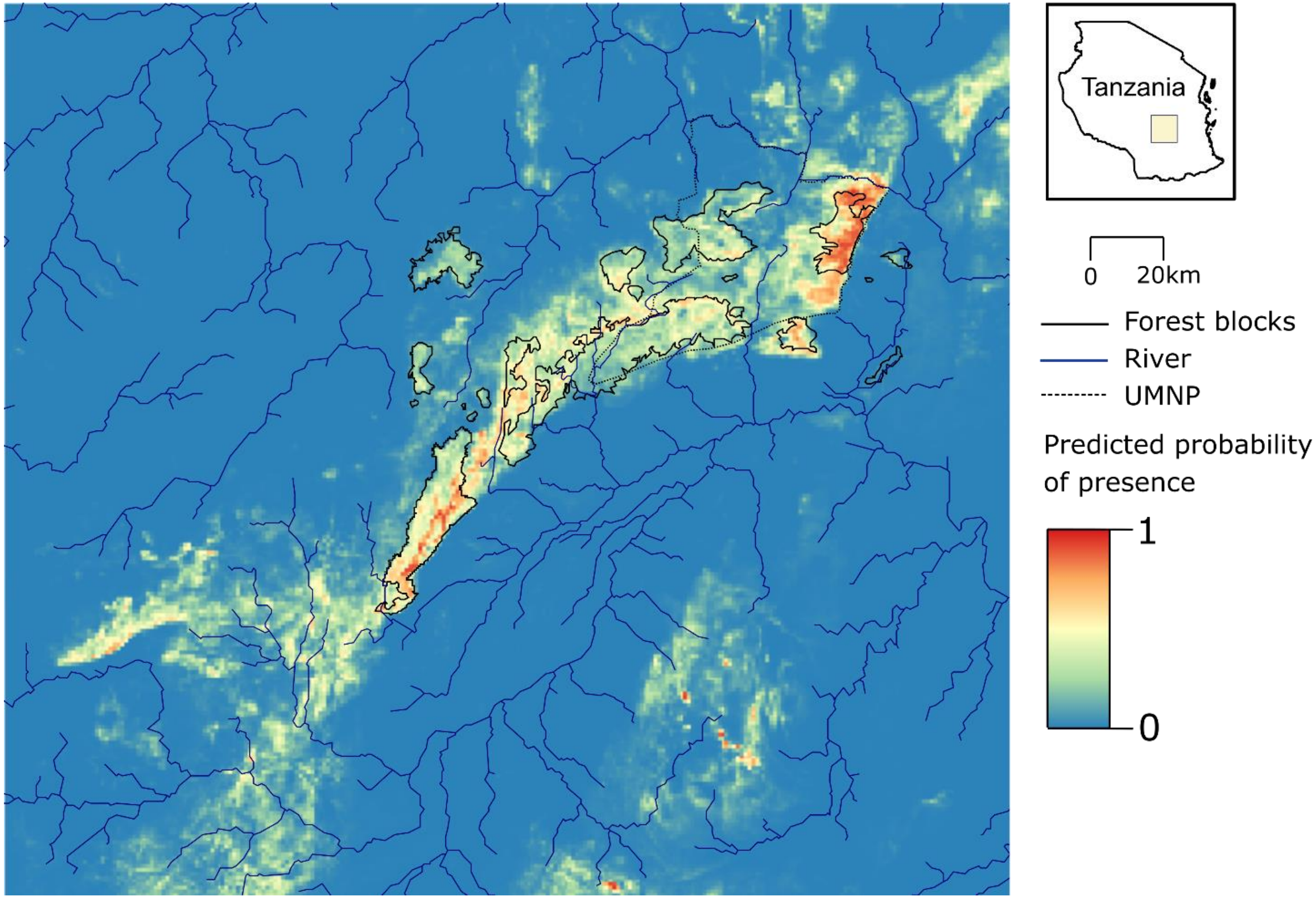

Figure 5.3. Current estimated predicted probability presence for the Sanje mangabey (*Cercocebus sanjei*) within the Udzungwa Mountains region in south-central Tanzania. Ecological Niche Modelling was performed using a Maximum Entropy Model in Maxent with a minimum training presence threshold, therefore a value of 0 (blue) represents absence and 1 representing the highest probability of presence.

5.3.2. Population Habitat and Viability Assessment

Mortality Parameters Sensitivity Testing

The test for the impact of increasing and decreasing mortality rates for different sex and age classes found that this significantly impacted the growth rate of the population when considering mortality of adult females (R^2=1.00; $p < 0.01$), subadult females (R^2=0.98; $p < 0.01$), juvenile females (R^2=0.96; $p < 0.01$), and infant males (R^2=0.97; $p < 0.01$) and females (R^2=1.00; $p < 0.01$; Figure 5.4).

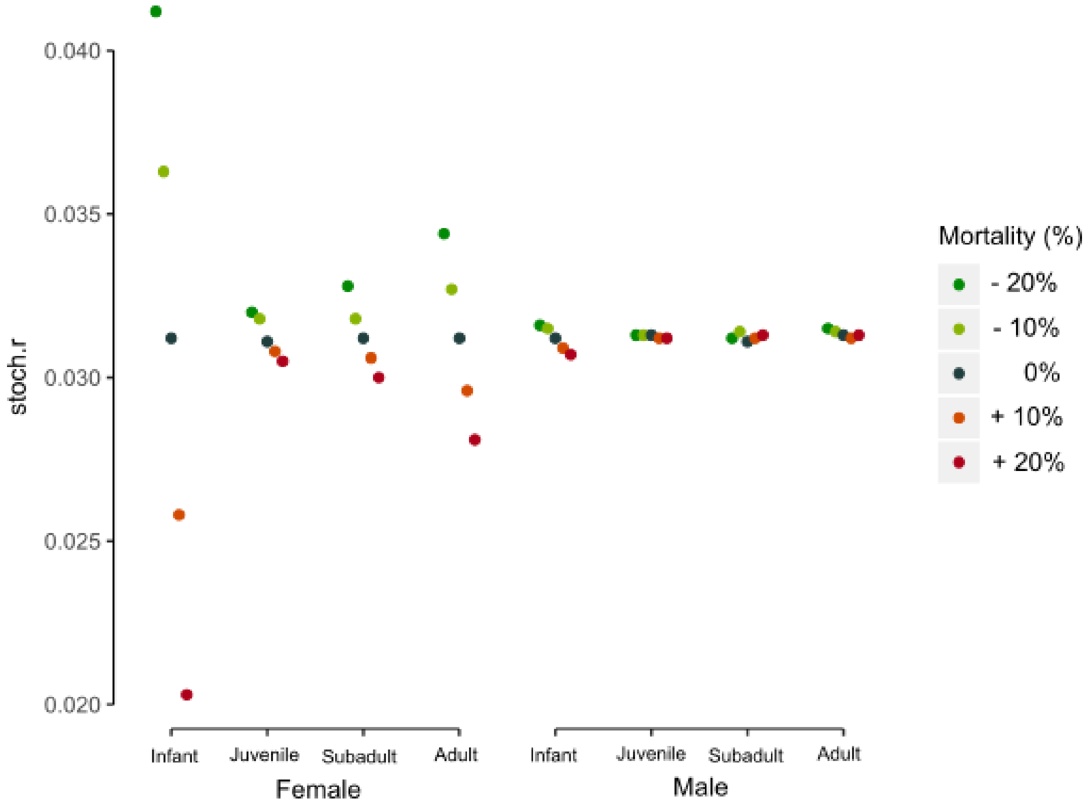

Figure 5.4. Sensitivity testing for the mortality rates used for different age and sex classes for a test model population of the Sanje mangabey of 500 individuals and a carrying capacity of 1000 individuals, for 1000 iterations. The sensitivity tests used the baseline values (0%) compared to an increase in mortality by 10% and 20%, and a decrease in mortality by -10% and -20%.

Estimated Current Scenario with No Extinction Pressures

The Sanje mangabey baseline scenario without extinction pressures showed a positive stochastic growth rate for the metapopulation (r = 0.031) and populations individually (Mwanihana: r=0.029; Uzungwa Scarp: r=0.032). The Mwanihana population asymptotes to the carrying capacity after ~10 years and remains stable at this population size up to 100 years (Figure 5.5). The Uzungwa Scarp population asymptotes to the carrying capacity after ~25 years and remains stable at this size up to 100 years.

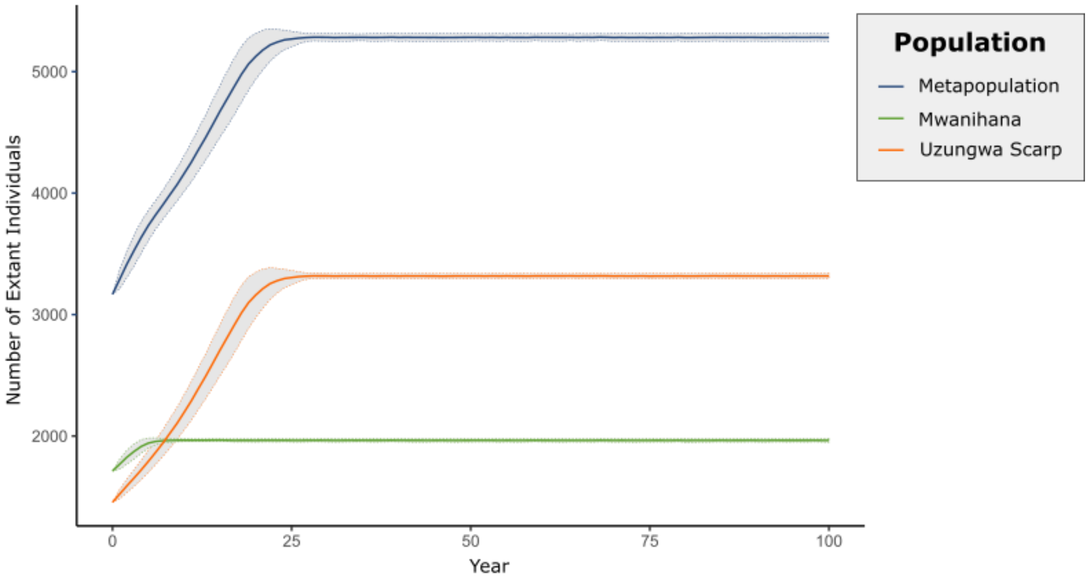

Figure 5.5. Baseline scenario for the Sanje mangabey (metapopulation: blue) and its two populations in the Udzungwa Mountains, Tanzania: Mwanihana (green), Uzungwa Scarp (orange). The solid line represents the mean average and the grey shaded area indicates the standard deviation.

Extinction Pressure Sensitivity Testing

The threat of poaching modelled as an individual threat showed no significant effect on the final extant population size or the probability of extinction, with no models showing any probability of extinction. However, with increasing poaching, the heterozygosity of the metapopulation significantly decreased ($p < 0.01$) compared to the baseline with no poaching, with additional 2% mortality due to poaching in the Uzungwa Scarp.

The threat of deforestation, by loss of carrying capacity, showed a significant effect (all $p < 0.01$) in decreasing the metapopulation extant population size after 100 years for all levels of loss modelled (0.1% loss per year up to 1% loss per year). Equally, in each forest, the extant populations decreased significantly in the corresponding forest with all scenarios of decreasing carrying capacity modelled. Heterozygosity declined significantly with all scenarios of decreases in carrying capacity modelled ($p<0.01$). Heterozygosity decreased in each forest individually when carrying capacity was a loss of 0.5% per year and greater in each ($p<0.01$). The number of alleles in Mwanihana, Uzungwa Scarp and the metapopulation significantly decreased with any decrease in carrying capacity in either forest ($p<0.01$). The stochastic growth rate ($r=-0.042$; $p=0.04$) was estimated to significantly decrease in the metapopulation from the baseline scenario when carrying capacity decreased by 1% in both forests at the same time.

When combining extinction pressures, scenarios where values of K were included showed the greatest impact on the number of extant individuals in Mwanihana (Figure 5.6). In the Uzungwa Scarp, poaching impacts at +1% and +2% mortality increases slowed the growth rate of the population, however declines in K contributed most to the decreasing population size, producing a gradual decline at losing 0.1% K/year, and a sharp decline losing 0.5% K/year (Figure 5.6).

Estimated Current Scenario with No Intervention

The model of the estimated current scenario with extinction pressures found both populations were limited by carrying capacity. This allowed only minimal growth before the population showed a gradual decline in response to the remaining pressures of decreasing carrying capacity with deforestation/degradation, poaching and to disease (Figure 5.7a). The stochastic growth rate (Figure 5.7b) and heterozygosity (Figure 5.7c) is estimated to decline slightly for both populations, however the number of alleles was estimated to significantly decline (Metapopulation: 0 years = 6334, 100 years = 931; Mwanihana: 0 years = 3424, 100 years = 437; Uzungwa Scarp: 0 years = 2910, 100 years = 494; Figure 5.7).

Conservation Management Scenarios

Increasing the carrying capacity of each forest by 0.005% per year resulted in much larger population sizes (number of extant individuals) after 100 years, with Mwanihana increasing by 11.48% and the Uzungwa Scarp population increasing by 98.27% compared to the scenario of no intervention (Table 5.7). The stochastic growth rate in Uzungwa Scarp increased by 9.83% whilst Mwanihana remained relatively similar to the current scenario (-0.83%; Table 5.7). For both populations, the proportion of heterozygosity retained increased, the number of alleles increased, and inbreeding decreased (Table 5.7), however this result is an artefact of the VORTEX model (see Discussion).

With poaching removed from the Uzungwa Scarp, the stochastic growth rate increased from the no intervention scenario by 31.21%, and the proportion of heterozygosity retained and number of alleles increased by a small amount (Table 5.7). However, this change had little effect on the number of extant individuals after 100 years, likely carrying capacity being the limiting factor for population size growth. Combining both approaches, increasing carrying capacity and eliminating poaching in the Uzungwa Scarp resulted in increased population sizes, stochastic growth rate, proportion of heterozygosity retained, and number of alleles, and a decrease in inbreeding for both populations (Table 5.7). In the Uzungwa Scarp allowing for increased carrying capacity alongside eliminating poaching resulted in the population size

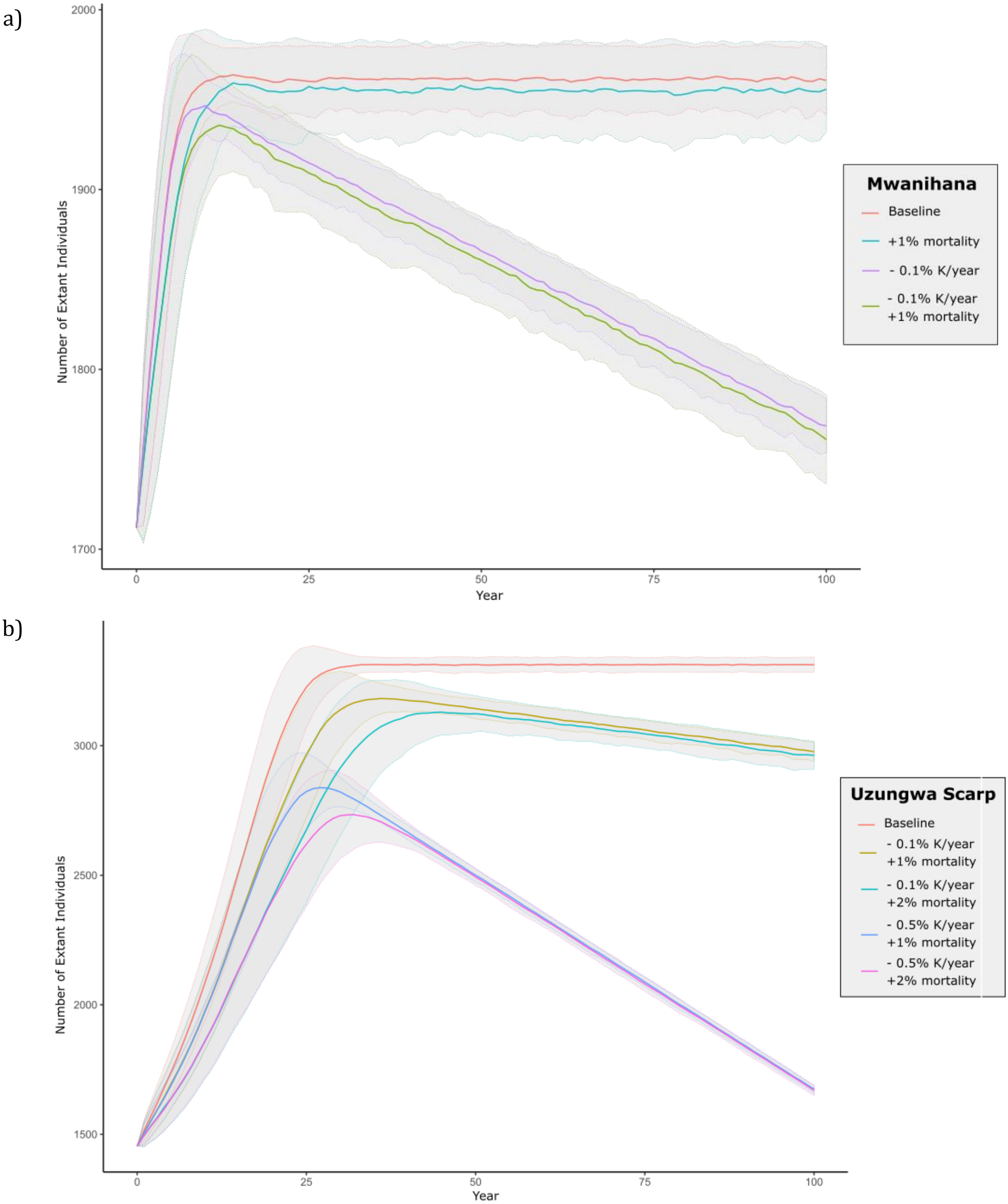

Figure 5.6. Modelling the impact on number of extant individuals of varying extinction pressures estimated for each population of the Sanje mangabey in a) Mwanihana and b) Uzungwa Scarp over 100 years from the current estimated scenario. With loss of carrying capacity, the populations show deterministic declines with the yearly loss. The solid line represents the mean average and the grey shading shows the standard deviation.

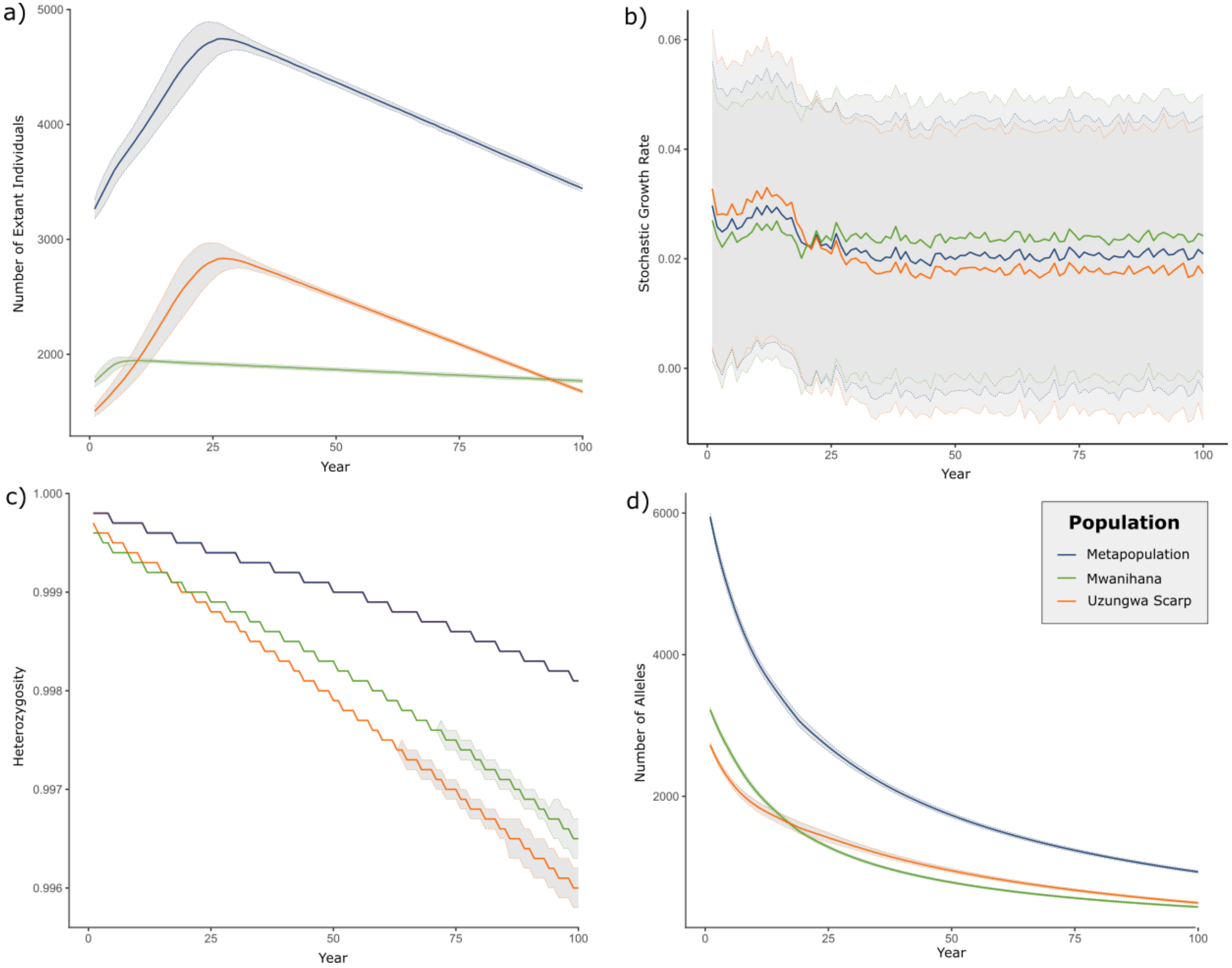

Figure 5.7. Predicted life history parameter change: a) extant individuals, b) stochastic growth rate, c) heterozygosity, d) number of alleles, over the next 100 years for the Sanje mangabey (*Cercocebus sanjei*) with no intervention and estimated current conditions continue. The solid line represents the mean average and the grey shading shows the standard deviation.

increasing by 98.86%, compared to only 0.06% increase by eliminating poaching only. Additionally, this combination of measures allowed for an increased stochastic growth rate by 41.62% compared to only a 31.21% increase in the eliminating poaching only model and 9.83% increase in the carrying capacity only model.

With the results of the ENM showing potentially suitable habitat in New Dabaga, Luhombero and areas of Matundu, the introduction of a small group of individuals (n=15) was modelled to Luhombero. When this measure was combined with increased carrying capacity and eliminating poaching across all populations, the metapopulation showed a high percentage increase in population size and stochastic growth after 100 years, however as the new population has limited genetic input, the metapopulation decreases in average heterozygosity and increased inbreeding (Table 5.7).

5.4. DISCUSSION

This study conducted the first population and habitat viability assessment of the Sanje mangabey, collating life history data collected from a habituated group and data from closely related species, with ecological niche modelling to identify key habitat structure and regions in the Udzungwa Mountains. This is, to our knowledge, the first population modelling exercise for a *Cercocebus* species since the assessment of the Tana River mangabey 30 years prior to this study (Kinnaird and Brien 1991). Modelling for the benefit of conservation management planning is a valuable tool to create informed actions, tailored to a species and its habitat. As an Endangered species isolated into two forest fragments, and with evolutionarily significant differences between each population, the results of the modelling potentially provide key information for the conservation management planning of the species. Already putatively considered to be in decline, without intervention, the Sanje mangabey populations are expected to continue to decline with the current levels of pressure.

5.4.1. Ecological Niche Model (ENM)

In the ENM for the current scenario, the parameter with the greatest percent contribution and permutation importance, significantly higher than all other parameters, was the compound topographic index. This parameter indicates soil moisture combined with the topography and catchment areas. The Sanje mangabey was found to sharply increase in presence probability when the compound topographic index was low, indicating a preference to regions with small catchment areas and steep slopes, consistent with the known distribution in montane forests. High compound topographic index values are

Table 5.7. Estimates for key parameters for the estimated current scenario and each conservation management plan modelled for the Sanje mangabey (*Cercocebus sanjei*) after 100 years: i) increase in carrying capacity (K), ii) decrease in poaching, iii) combined increase in K and decrease in poaching, iv) translocation of 15 individuals from Uzungwa Scarp to a new forest Luhombero with supplementation of four adult females and one adult male every 20 years, v) all measures combined in a mixed approach. The percentage change for each conservation scenario is shown as comparison to the mean average from the current scenario with no intervention.

Scenario	Pop.	Nextant ±SD	% change	r.stoch ± SD	% change	Het ± SD	% change	Nalleles ± SD	% change	Inbr ± SD	% change
Current	MW	1768 ±16	-	0.0242 ±0.026	-	0.996	-	438 ±11.8	-	0.0032 ±0.001	-
	US	1674 ±14	-	0.0173 ±0.027	-	0.9965	-	494 ±13.9	-	0.0027 ±0.001	-
	Meta	3441 ±26	-	0.0209 ±0.025	-	0.9981	-	932 ±18.2	-	0.003 ±0.001	-
↑ K	MW	1971 ±19	+11.48	0.024 ±0.025	-0.83	0.9962	+0.02	464 ±12	+5.92	0.0031	-3.13
	US	3319 ±44	+98.27	0.019 ±0.025	+9.83	0.9974	+0.09	673 ±19.2	+36.14	0.0023	-14.81
	Meta	5290 ±58	+53.73	0.0208 ±0.024	-0.48	0.9984	+0.03	1136 ±22	+21.94	0.0025	-16.67
↓ poaching	MW	1767 ±16	-0.06	0.0238 ±0.026	-1.65	0.996	0.00	438 ±11	-0.03	0.0032 ±0.001	0.00
	US	1675 ±13	+0.06	0.0227 ±0.027	+31.21	0.997	+0.05	503 ±13.5	+1.79	0.0027 ±0.001	0.00
	Meta	3442 ±34	+0.029	0.0233 ±0.026	+11.48	0.998	-0.01	941 ±17.3	+0.94	0.003	0.00
↑ K & ↓ poaching	MW	1971 ±19	+11.48	0.0243 ±0.026	+0.41	0.9962	+0.02	464 ±11.9	+6.03	0.0031 ±0.001	-3.13
	US	3329 ±28	+98.86	0.0245 ±0.026	+41.62	0.9974	+0.09	691 ±17.7	+39.85	0.0021	-22.22
	Meta	5301 ±42	+54.05	0.0244 ±0.025	+16.75	0.9985	+0.04	1155 ±21.8	+23.97	0.0025	-16.67
↑ K, ↓ poaching & new population	MW	2059 ±19	+16.46	0.0248 ±0.025	+2.48	0.9963	+0.03	474 ±12.9	+8.42	0.003 ±0.001	-6.25
	US	3474 ±35	+107.53	0.023 ±0.025	+32.95	0.9975	+0.10	706 ±17.6	+42.78	0.0021	-22.22
	LU	3267 ± 596	-	0.0357 ±0.027	-	0.9665	-	128 ±7.9	-	0.0332 ±0.006	-
	Meta	8800 ±603	+155.74	0.0278 ±0.025	+33.01	0.9948	-0.33	1263 ±22.9	+35.54	0.0138 ±0.003	+360.00

Populations: Mwanihana (MW), Uzungwa Scarp (US), Luhombero (LU), and the metapopulation (Meta).

representative of gentle slopes or plains and areas with large catchments, reflecting the lower elevation regions in the Udzungwa Mountains between forest fragments, areas that the mangabeys are observed to avoid (Rovero *et al.* 2017). The parameter with the second greatest percent contribution and permutation importance was precipitation seasonality (Bio15) with presence probability highest for low levels of precipitation seasonality, representative of regions of greater seasonal stability. Increased brightness, the parameter with the third highest contribution to the model, was linked to increased presence probability. Brightness can be linked to soil cover and forest condition or/in addition, it can be linked to topographic variation (Cohen and Goward 2004). In an ENM study of chimpanzee (*Pan troglodytes verus*) presence probability, low brightness values were found in steep savannah regions, therefore more strongly affected by topography than forest condition in these regions (Fitzgerald *et al.* 2018). The results therefore need to be taken with caution however, considering the strong importance of compound topographic index as a parameter, it is most likely linked to the importance of topographic variation for the presence of Sanje mangabey.

The combination of these top three contributing factors to the ENM for the Sanje mangabey further supports the general consensus that low elevation forest corridors may not be a viable conservation measure for the Sanje mangabey, with preference for montane regions and distance from the forest edge (Rovero *et al.* 2017), and exhibit avoidance behaviour from baboons (*Papio* spp.) found predominantly at lower elevations in the region (discussed in Chapter Three). Further, the stability of seasonal precipitation as an important factor is reflective of the high levels of endemism in the Eastern Arc maintained in the montane forest 'islands' due to the stability of these habitats during periods of climatic change in surrounding regions (Marchant *et al.* 2007; Mumbi *et al.* 2008; Fjeldsa and Lovett 1997). This stability has allowed the Sanje mangabey to speciate and persist in these forests over time (the two populations diverging ~0.7 MYA; Chapter Three). Therefore, it would be most suitable to direct conservation management strategies (such as increase the carrying capacity available to the populations) in montane forest regions with low compound topographic index values, steepness of terrain, and more stable precipitation across seasons.

These considerations were taken across into the population models in this study, focussing on increased carrying capacity, both as forest regeneration for each population's habitat and by introducing individuals to a new habitat estimated to be suitable by this ENM model. In the region considered by this study, the ENM estimated three primary forests as potentially suitable for presence of the Sanje mangabey: New Dabaga, Matundu and Luhombero (forest locations: Figure 5.2; ENM: Figure 5.3). Luhombero was modelled as the example forest as this forest most isolated from human settlements and lies mostly within the UMNP, with the greatest chance of receiving a high-level protection out of these forests. However, the information is limited in the ENM and ground truthing of the habitat vegetation and diet resources would be required to further identify whether this forest is suitable. The Uzungwa Scarp was chosen to supplement this population with the aim of preserving the higher genetic diversity found in this population (Chapter Four) and as the more threatened option for the source population. Additionally, the Udzungwa red colobus, black-and-white colobus, and Sykes' monkey populate Luhombero and are species sympatric to the Sanje mangabey in both populations. The kipunji is also present in Luhombero (Ehardt and Butynski 2006), the only forest it is found within the Udzungwa Mountains, suggesting the forest is suitable for sustaining viable isolated populations of a limited size and with no chance of dispersal and gene flow.

5.4.2. Stochastic Population Modelling

No models in this study predicted extinction in the next 100 years with the parameters tested, reflecting the stability of the populations to changing conditions estimated in the demographic history and historic ENM based on bioclimatic variables in Chapter Four. Data available for the stochastic models was limited. Data on the life history of the Sanje mangabey is being developed by the Sanje Mangabey Project (Fernández, Ehardt, *et al.* 2019), and thus, the parameters in this study were collated from previous studies of the Sanje mangabey and gaps were filled by the closest species that could be found with suitable data to include. Therefore, the results and accuracy of the model reflect the current gaps in knowledge of the species. It would be beneficial for the species to study mortality rates of different age classes (data from *C. galeritus* used in this study) as, depending on sex and age, mortality of certain groups, such as infant and adult females, have a significant influence on growth rate (see Figure 5.4). To improve the species-specific data available for the models, the development and study of more habituated (and, if possible, wild) groups throughout the full distribution of the Sanje mangabey is needed. Currently habituated groups are only present in the southern region of Mwanihana forest. This region is very well-protected with the more frequent presence of rangers and tourists than in the northern region of the forest and areas deeper into the forest, furthest from the main road. Therefore, groups may not fare as well, and life history data may vary with the greater disturbance estimated in that region. More importantly, no groups have been studied in the Uzungwa Scarp due to the greater logistical restraints and difficulties of studying in that forest compared to Mwanihana. A vital knowledge gap remains in the behaviour and life history of this genetically diverged population and its social groups in the lesser protected and far more disturbed Uzungwa Scarp forest. Comparing the data collected from each population would improve our understanding of the species and whether previous genetic differentiation found between the two populations (Chapter Three; Chapter Four) is reflected in individual and group behaviour.

If no intervention was made in the current estimated scenario, the range of poaching levels modelled had little effect on population viability apart from slowing the time for populations reaching carrying capacity (Figure 5.6). This is likely due to the very low level impact of poaching modelled that aimed to reflect that the Sanje mangabey is not considered the primary target of poaching, but instead threatened as by-catch (Rovero *et al.* 2009). The impact of hunting on the Sanje mangabey has not been previously quantified. However the significantly lower group density (Paddock *et al.* 2020) and occupancy (Hegerl *et al.* 2017) of the Sanje mangabey in the Uzungwa Scarp, in line with the differences between these forests for other species (Oberosler *et al.* 2019), is thought to reflect that the Sanje mangabey is affected by the same threats, including poaching. Further study to estimate the impact of hunting on the Sanje mangabey is required to fully understand its impact.

All scenarios that included decreasing carrying capacity showed a large decrease in number of extant individuals after 100 years, deterministically reflecting the rate of decrease modelled for carrying capacity (Figure 5.6). This is because the populations are at carrying capacity and their intrinsic biological parameters would lead the population to continue to grow at 3.5% per annum in the absence of carrying capacity constraints. For the estimated current scenario after 100 years, the population showed a declining number of extant individuals, proportion of heterozygosity retained and in growth rate (Figure 5.7). The conservation management approaches modelled all showed promise for improving the viability of each population, however when increasing carrying capacity was included as an action, improvements to estimated viability were overall the most successful approaches. Reversing the rates of loss of carrying capacity and instead increasing habitat by 0.005% each year and eliminating the additional mortality of adults in Uzungwa Scarp due to poaching enabled each population to thrive. This was the only model where all viability indicators measured (number of extant individuals, stochastic growth rate, heterozygosity, number of alleles, and inbreeding) improved after 100 years, and improved substantially. Sanje mangabeys are considered to be flexible in habitat use, using both primary and secondary forests (McCabe and Emery Thompson 2013), so would be predicted that the populations would be able to benefit relatively quickly from the regenerating forest. However, it is very important to note that the inadequate way that Vortex models genetic diversity (all new individuals born carry two new alleles at a hypothetical genetic locus) means that positive responses in genetic diversity parameters do not reflect reality. In practice, increases in genetic diversity are either brought about by mutation (which would require far larger population sizes than here to be significant) or by gene-flow, which, as already established, is impossible in reality (and may not be desirable as the two populations represent two ESUs).

When modelling the creation of a new, third population in Luhombero (Figure 5.2), with increased carrying capacity for the metapopulation, the number of extant individuals and stochastic growth rate could both increase. However, due to creating a population bottleneck by limiting the number of founders to only 15 individuals, the heterozygosity and inbreeding rates would be high for this population. As in the PHVA conducted for the Tana River mangabey, the social dynamics that would result from this introduction of individuals and translocation of individuals to supplement the population in the future would be unknown (Kinnaird and Brien 1991). Therefore, combining the unknown ecological consequences and this limited genetic diversity, this scenario would only be considered in extreme circumstances, assuming most likely this would be in the case of a significant decrease in the viability of one of the two extant Sanje mangabey populations.

The levels of inbreeding and whether this is present or not needs to be assessed in individual populations as this was not included in the models presented here. In Chapter Three, no recent demographic expansion or contraction was found using Bayesian Skyline Plot, Tajima's D or Fu's F_s analyses, therefore estimated the female effective population sizes have remained stable for a long period of time. The stable populations of these descendants may be representative of a limited gene pool and generations of highly related individuals mating, similar to the evolutionary history of African cheetah (*Acinonyx jubatus*; O'Brien *et al.* 2017). However, the significant levels of mitochondrial genetic diversity uncovered in Chapter Four would suggest that very low genetic diversity and inbreeding is unlikely. Genomic data can be incorporated into stochastic vortex models, which provides invaluable insight when considering small, isolated populations that are at the greatest threat to detrimental genetic processes. The viability of populations can be influenced by loss of genetic diversity and adaptive potential, inbreeding depression, and genetic drift. For example, considering the impact of inbreeding depression, Bruford *et al.* (2010) identified a mixed management conservation approach for fragmented orangutan populations in the Lower Kinabatangan Wildlife Sanctuary, Malaysia, that would minimise both inbreeding and extinction risks, in which the measures individually would not have achieved. The Sanje mangabey conservation management models would further benefit from the inclusion of genome-wide data to further understand this aspect of the species' viability and response to action.

5.4.3. Conservation Recommendations

Following the combination of ENM and PHVA model results two key recommendations would be made, one preventative and one active conservation measure: i) increase the protection of the Uzungwa Scarp, and ii) regenerate and increase available habitat in montane regions for both populations. Firstly, the priority action would be to increase the protection of the Uzungwa Scarp to reduce both forest degradation and poaching, to protect and prevent further decline of the carrying capacity and population size in this evolutionarily significant unit. Camera trapping results over eight years in Mwanihana found a decline in poaching with a decreasing number of snares collected over this time, and overall protection is sustaining mammal species in the forest, and therefore the National Park regulations were found to be effective and worth the investment (Oberosler *et al.* 2020). As discussed previously, the protection in Mwanihana is significantly higher than in the Uzungwa Scarp, reported as 78 versus one permanent staff member respectively, with 60 of the 78 in Mwanihana involved in active patrols (Hegerl *et al.* 2017). Active enforcement has increased in the Uzungwa Scarp with the upgrade from Forest Reserve to Nature Reserve status in 2017, however further protection is required to improve the viability of the species within the forest. This may be achieved by further increasing the protection of the Uzungwa Scarp, potentially under National Park regulations that have proven effective for Mwanihana, and therefore decreasing the disparity in protection between the Uzungwa Scarp and Mwanihana. Following this to further improve the viability of the two population, we recommend forest regeneration and increased carrying capacity in both forests at higher elevation montane forest regions to improve the potential of population growth for both populations. Further investigation into the preference of the Sanje mangabey of dietary items and habitat composition is required to identify habitat suitability to a greater resolution within the forests. From behavioural observations that found the species to avoid both human disturbance and baboons at lower elevations, and ecological niche modelling in this study identifying a preference for steep montane forests, current efforts to restore forest corridors (such as the Mngeta corridor between Uzungwa Scarp and Matundu; Rovero and Jones 2012) are unlikely to be a successful conservation action for the Sanje mangabey.

Additional to the threats modelled in this study, development of the main road that follows the eastern boundary of Mwanihana forest is expected to increase human encroachment in the region. Therefore the creation of a buffer zone to protect the forest edge at lower elevations, as discussed by Cavada *et al.* (2019) and Oberosler *et al.* (2020), will be critical in protecting the forest at higher elevations where the mangabey is found. Finally, these conservation actions cannot be successful without engaging local stakeholders. Work by local conservation groups, such as the Sanje Mangabey Project, Udzungwa Ecological Monitoring Centre and the Udzungwa Forest Project, needs to continue working with local communities to raise awareness (see Fernández *et al.* 2019) and offer alternative livelihoods to poaching, such as working within monitoring and patrolling initiatives.

5.5. ACKNOWLEDGEMENTS

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CHAPTER SIX

General Discussion

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6.1 General Discussion

The work in this thesis has added to the current understanding of the Sanje mangabey to allow for informed conservation action planning. Priorities to develop our knowledge of the species were identified prior to the study as: i) estimating the current population size, and ii) investigating the genetic diversity and structure of the species (Fernández *et al.* 2019). The results of the work conducted within this thesis will directly contribute to informing conservation actions. These recommendations will be published in the Mangadrill Conservation Action Plan and will be presented at international conferences and to local stakeholders involved in policy making. Five studies were designed to address these knowledge gaps, one of which was not successful and thus not included within this thesis (discussed later). Here, a summary of the key results and achievements of each chapter is described:

Chapter Two: Population Survey Paddock *et al.* (2020)

In this study I estimated the population size of the Sanje mangabey for the first time, using an alternative method to the traditional visual transect surveys used previously for primates in the Udzungwa Mountains. I designed an acoustic point transect survey using distance modelling to address the issues of observing and counting the mangabeys in dense habitat and with their tendency to flee quickly when disturbed. The method applied successfully detected mangabey groups using the distinctive 'whoop-gobble' call and estimated a population size that reflected previous estimates considered most accurate. A total of 3,167 (95% CI: 2,181-4,596) individuals were estimated, 1,712 (95% CI: 1,141-2,567) individuals in Mwanihana and 1,455 (95% CI: 783-2,702) in Uzungwa Scarp, with a significantly lower group density in the Uzungwa Scarp (0.15 groups/km^2; 95% CI: 0.08-0.27) than in Mwanihana (0.29 groups/km^2; 95% CI: 0.19-0.43; Student's *t*-test: $t = 2.25$; $df = 26$; $p = 0.03$). The results of this study confirmed previous predictions that the Uzungwa Scarp is more highly impacted from habitat degradation and human encroachment, with this difference in group density potentially reflective of the differing levels of formal protection. This chapter has provided the first systematic estimate of population size for the Sanje mangabey. This has contributed key data for consideration in planning conservation actions, highlighting the need to prioritise increasing protection of the Uzungwa Scarp. Future recommendations of how to develop this acoustic method for greater accuracy were also described, which has offered insight for monitoring the Sanje mangabey populations in the future and a framework for studies of other elusive primates (e.g., Linder *et al.* 2021).

Chapter Three: Phylogenetics

This chapter aimed to investigate the phylogenetic placement of the Sanje mangabey within the *Cercocebus* phylogeny to address an existing knowledge gap. Outstanding questions were whether the Sanje mangabey was a distinct species or was part of a group of *Cercocebus galeritus* subspecies, alongside the Tana River (*C. [galeritus] galeritus*), agile (*C. [galeritus] agilis)* and golden-bellied mangabeys (*C. [galeritus] chrysogaster*). No previous study had included the Sanje mangabey within phylogenetic analyses, as no genetic data were available previously and no type specimen was available for morphological analyses. The phylogeny of the *Cercocebus-Mandrillus* clade was reconstructed using concatenated fragments from two nuclear (autosomal CD4 gene and Y chromosomal testis-specific protein TSPY), and two mitochondrial fragments (cytochrome oxidase subunit II: COII; and control region: CR). These fragments were used as they represented three inheritance pathways: autosomal, paternal, and maternal. This chapter produced a surprising result, with the Sanje mangabey estimated to be an evolutionarily distinct lineage from these other three species, diverging 2.34 million years ago (MYA; 95% HPD interval: 1.73 – 2.95 MYA). A second surprising result was that the two populations represent two distinct evolutionary lineages, diverging 0.77 MYA (95% HPD interval: 0.43 – 1.15 MYA), a more ancient date than the three other mangabey species diverged from one another. These results have provided a critical insight into the evolutionary history of the Sanje mangabey, making protection of each population as a distinct unit a conservation priority. As a result of this research, it was concluded that the two populations should be considered preliminary evolutionary significant units (ESUs), pending further investigation with nuclear, genome-wide markers.

Chapter Four: Phylogeography

Following on from the results in Chapter Three, further investigation into mitochondrial DNA (mtDNA) diversity and structure of multiple individuals from each population was conducted. This study aimed to estimate diversity and demographic history within the species. Alongside this, I aimed to estimate the impact of habitat suitability and the changing climate over the last ~140,000 years, using an ecological niche model (ENM). Estimation of suitable habitat was made for the current scenario, using presence points from the survey in Chapter Two, and extrapolated to three historic time points: i) Mid-Holocene (6,000 YA), ii) Last Glacial Maximum (22,000 YA), and iii) Last Interglacial Maximum (LIG; 120,000-140,000 YA). The study amplified a 423 bp fragment of the mtDNA control region using newly designed primers to sequence two overlapping mtDNA fragments, amplified from DNA extracted from faecal samples, in the Sanje mangabey. Sequences were produced and analysed for 36 individuals from Mwanihana and 28 from Uzungwa Scarp. These primers can be used in future studies to include more individuals in the sampling or to compare diversity over time in response to disturbances, extreme events, or conservation measures. The study corroborated the result in Chapter Three with genetic differentiation found to be highly significant (F_{ST}= 0.95, $p<0.0001$) between the populations, with reciprocally monophyletic mtDNA haplotypes separated by a minimum 16 segregating substitutions. Combining the results of the estimated stable demographic history for each population and the ENM showing probable suitable habitat in montane areas with more stable climatic conditions across time, suggests that the populations diverged on the ancient timescale in Chapter Three and then speciated in these stable montane forest 'islands'. The results of this chapter further highlight the genetic distinctiveness of each population and the need for conservation planning and future genetic studies based in nuclear/genomic data to consider this.

Chapter Five: Population Viability

The final data chapter aimed to estimate the viability of each population. In this study, all existing information of the Sanje mangabey's life history and threats were collated, to estimate the viability of each population and test the effect of different conservation management scenarios. This was achieved using VORTEX simulations (Lacy *et al.* 2018) to estimate the current scenario and the response of the populations to different conservation approaches over the next 100 years. Alongside this, a current scenario ENM was run with bioclimatic variables (as in Chapter Four) and biophysical variables within the Udzungwa Mountains for a more detailed insight into the correlating variables with Sanje mangabey presence locations.

The ENM estimated that, from the set of variables used, the greatest contributing variable was the compound topographic index of the habitat. Sanje mangabey presence probability was higher in regions with low catchment areas (montane regions) and steep slopes, which are consistent with montane regions in the Udzungwa Mountains. The viability models with no intervention showed the populations are estimated to decline in population size, deterministically with the declining carrying capacity over the next 100 years. The most successful conservation actions modelled for both populations were those where carrying capacity was increased, modelling forest protection and regeneration. With the results of the ENM, it was concluded that it would be best to regenerate forest in higher elevation montane forest regions within the current forest blocks. The models in this chapter have directly impacted the conservation recommendations made in this General Discussion, and therefore will provide insight for the Sanje mangabey conservation actions being developed for the Mangadrill Conservation Action Plan (Fernández *et al.* 2019).

6.2 Conservation Genomics

The aims of this thesis originally included generating genome-wide SNP data to assess the genetic diversity and structure of the two populations, and to compare this to the mitochondrial phylogeographic results in Chapter Four. The use of SNPs in conservation genomics is discussed here and a brief overview of the study that was conducted, but not included in this thesis.

Markers used to determine the genetic diversity and structure of populations have developed since the first studies of population genetics, including mitochondrial DNA (mtDNA; as in Chapter Four), microsatellites, and single nucleotide polymorphisms (SNPs; generated by a wide range of sequencing methods), and those used much less frequently in recent studies, such as allozymes, restriction fragment length polymorphism (RFLP) and random amplified polymorphic DNA (RAPD; Forcina and Leonard 2020). Neutral markers and adaptive variation have not been found to show similar patterns in response to changes in population demographics, therefore combining the analysis of both regions of the genome can help infer a more accurate demographic history (Teixeira and Huber 2020).

The generation of SNPs through Next Generation Sequencing (NGS) allows the genotyping of thousands of loci compared to the low double figures with methods such as microsatellites, for often similar amounts of workload and, now, a lower per-locus cost with NGS (Funk *et al.* 2012). Comparing SNPs to microsatellites, not only do SNPs allow the study of adaptive patterns, they also benefit from methods being replicable and results being comparable between laboratories and the generated data openly available in online, open access databases for future studies.

6.2.1 Double-Digest Restriction Site-Associated DNA Sequencing

Sampling of faeces, as a non-invasive method, is a preferable as a source of host DNA in conservation genomics. However, these samples are limited as they consist of short and degraded DNA fragments, and have low host DNA concentration whilst high in exogenous DNA concentration (Costa *et al.* 2017). DNA extracts from faecal samples collected from wild baboons were found to have only an average of 0.6% (range: <0.01-4.9%) host DNA (Chiou and Bergey 2018). The majority of exogenous DNA extracted from faecal samples represents the gut content and will also include compounds that can act as PCR inhibitors (Monteiro *et al.* 1997; Scott and Playford 1996). At present, sequencing directly from faecal DNA extracts would require an excessive number of sequencing lanes and major expense to achieve reasonable coverage of the host genome alongside exogenous DNA (Perry *et al.* 2010), therefore both increasing the host DNA concentration and using reduced representation sequencing (RRS) would allow for economical and practical genome-wide sequencing from faecal samples.

Several methods have been published for generating genomic data from faecal samples, including using microfluidic SNP arrays (von Thaden *et al.* 2017), RNA bait capture (e.g. Kohn, 2010; Perry et al., 2010) and restriction-site associated DNA sequencing (RADseq; Chiou and Bergey 2018; Chiou *et al.* 2019). Arrays and bait capture methods have proven successful; however, the protocols are expensive and the design requires a reference genome or high-quality samples (e.g. blood or tissue) to sequence the genome of the target species. A study comparing the results of RRS (DArTseq: a restriction enzyme method similar to RADseq) with whole genome sequencing (WGS) for measuring breeding success in captive Tasmanian devil (*Sarcophilus harrisii*) found RRS to be a successful proxy for the WGS results (Wright *et al.* 2020).

To address the low concentration of host DNA in faecal DNA extracts, Chiou & Bergey (2018) developed the *FecalSeq* protocol which aimed to provide a low-cost option for genome-wide sequencing of non-model species. This was achieved by including an additional step prior to library preparations for double digest RADseq (ddRADseq) to enrich and increase the concentration of host DNA. Using a pre-made kit used for enriching samples for microbial DNA, a supernatant can be removed from the DNA extract that is enriched for host DNA (having removed the microbial DNA). This is conducted using methyl-CpG-binding domain (MBD) beads in the DNA extract that binds to the higher density of CpG-methylated regions in mammalian DNA than in microbial DNA (Chiou & Bergey, 2018). In the study, this enrichment increased the concentration of host DNA in samples on average by 318-fold (range: 4.3-2632.2), making this suitable for ensuring higher coverage of host DNA sequences, especially when combined with reduced representation sequencing (Chiou and Bergey 2018).

The FecalSeq method from Chiou and Bergey (2018) was extensively tested, by myself and in collaboration with both the Cardiff University Genome Hub, UK, and CIBIO-InBio, Portugal (development throughout 2018-2021). Unfortunately, no material from multiple versions of library preparation produced sequences, and time and financial restrictions prevented further investigation after extensive troubleshooting of all stages of the protocol. Collaboration with the research group that developed the method would be beneficial as it remains unknown why sequences could not be generated. The final assumption was that the very low quality and concentration of the Sanje mangabey samples, and potential high concentrations of PCR inhibitors affected the final library preparation PCR. Rainforest plants can consist of high concentrations of secondary compounds that can inhibit extraction and PCR amplification (Monteiro *et al.* 1997; Scott and Playford 1996). The Sanje mangabey faecal samples were most often whole intact seeds or grasses and the faecal sample for extraction was removed from the outside of this plant material (Paddock pers. obs.). Therefore, this is likely to have been the potential cause for the FecalSeq method being unsuccessful combined with the low concentration of endogenous DNA in the samples. Furthermore, since no museum samples were available, nor live specimens, we were unable to compare our results with better quality DNA of non-faecal origin.

6.2.2 Future Population Genomics Studies

Measuring local adaptation in wild populations provides information that could benefit conservation actions such as assisted gene flow, investigating disease vulnerability or resistance, managing captive breeding, identification of hybridisation events and informing genetic rescue interventions (Flanagan *et al.* 2018). Further, monitoring the frequency of identified adaptive alleles that contribute to increased fitness may provide a measure for the viability of a population and the success of conservation management plans (Shafer *et al.* 2015; Flanagan *et al.* 2018). A balance must be made when aiming to conserve adaptive variation that this is not at the cost of diversity across the genome. Gene-targeted approaches, conserving particular genes over maintaining genome-wide diversity, are considered to only be feasible in very few circumstances and recommended to only be considered if the loci are reflective of an urgent threat to the viability of the species (e.g. disease resistance or temperature tolerance) and that the loci have a high probability of achieving the phenotypic objective (Kardos and Shafer 2018). An example of where this has been considered is for the kakapo (*Strigops habroptilus*). All remaining individuals were genome sequenced in 2016 with the hope to identify genes associated with clutch size and hatching success, two viability traits that have reduced, likely as a response to inbreeding or disease susceptibility (Yong 2017; Kardos and Shafer 2018; Jamieson 2014)

Targeting specific genes with known adaptive functions or selective pressures to assess diversity can provide insight to a species' conservation status. One such region is the major histocompatibility complex (MHC). The MHC consists of linked genes that have been found to significantly contribute to the immune system in mammals (Sommer 2005) and therefore highly relevant to viability measurements and conservation. Teixeira and Huber (2020) have argued that a high genome-wide neutral diversity is not as important in population resilience, its ability to genetically adapt to changing environments, as claimed in many studies. Instead, they suggest that diversity and evolutionary history of specific functional loci, which may be distinct from the history of neutral variants, and the selection pressures on these loci are more important. For example, a recent study analysed the MHC variability in three *Eulemur* species and found a lower diversity within an isolated population and the loci related to immune responses to parasites, highlighting the need for focussed conservation efforts (Winter *et al.* 2019).

By characterising a fragment of the MHC for the Sanje mangabey, some knowledge gaps for the species can be addressed. A higher MHC diversity is generally linked with more viable populations and is an informative indicator for conservation genetics (Ujvari and Belov 2011). Comparing MHC diversity between closely related species can identify potential selective processes that have occurred in each lineage's history. For example, the bonobo (*Pan paniscus*) and chimpanzee (*Pan troglodytes*) shared a common ancestor 1 MYA, however the bonobo MHC class I diversity was found to be lower compared to chimpanzees and humans (Maibach *et al.* 2017). This difference was attributed to a possible selection process at these loci since the two species diverged, such as pathogen exposure, that would have changed allele frequencies by a selective sweep. A study of cane toads (*Rhinella marina*), an invasive species in Australia, found low genome-wide diversity following the bottleneck from introduction, however there was high diversity at loci considered to be under selection (Selechnik *et al.* 2019). This suggests that the introduced individuals could adapt to new environmental conditions, with high diversity in loci linked to heat resistance and dehydration, even when the effective population size was low.

A study is in progress as continuation from this thesis that will investigate the diversity and potential structuring between the two Sanje mangabey populations for a fragment of the MHC complex class II DQB gene. The protocol for this method has been developed for approximately 24 samples from each population, with each individual sample barcoded with multiplex identifier sequences, to be sequenced using an NGS platform. With the two Sanje mangabey populations diverging on an ancient timescale, testing for balancing selection can provide an insight into their evolutionary history and comparing levels of diversity may provide an insight into the viability of each population. With Sanje mangabeys prone to infections from tick bites, especially in infants in the early dry season (McCabe and Fernández 2018), this understanding of loci linked to parasite and pathogen resistance may play a crucial role in conservation management planning for each population. Although representing a limited portion of the nuclear genome, analysis of differentiation between the two populations at a locus that predominantly shows lower differentiation due to balancing selection will add further insight to the evolutionary distinctiveness of the two putative ESUs.

6.2.3 Integrating Conservation Genetics with Conservation Policy

Conservation of genetic diversity is essential to the viability of wild populations. Actively monitoring and actioning conservation plans to protect genetic has logistical constraints, often through financial and manpower limitations, but frequently in its inclusion in policy (Shafer *et al.* 2015).

For example, on an international scale, the Convention on Biological Diversity (CBD) is a framework that countries sign to commit to protecting biodiversity, including some recognition for protecting genetic diversity. However, many consider the CBD to be lacking in the importance put on genetic diversity, often focussing on ecosystem and species-level diversity rather than population level genetic diversity assessments, and the selection of species it aims to protect (Hoban *et al.* 2020). The CBD is not legally binding and individual countries are responsible for implementing a National Biodiversity Strategy and Action Plan (NBSAP). In 2010, of the plans available to view, one-third did not explicitly include a strategy for preserving genetic diversity and only one-fifth considered the need to monitor this diversity (Laikre 2010). Since the CBD was launched in 1992, the focus for preserving genetic diversity has been rooted in protecting species with commercial use, such as animal and plant species in agriculture and domesticated animals. The consequence of this is potential loss of genetic diversity in species that do not fall under these categories, and the fallout from the loss of the ecosystem services these species provide. In light of this, Hoban *et al.* (2020) argued the need for inclusion of conservation geneticists with the scientific experts informing policy, and for conservation geneticists to be proactive and ensure their research and its importance is presented in front of relevant stakeholders responsible for policy decision-making.

The International Union for Conservation of Nature (IUCN) Red List, the most recognised database for assessing the extinction risk of species, considers genetic factors in its assessments but does not use any measure of genetic diversity to estimate extinction risk (Garner *et al.* 2020). Concern is raised by the lack of genetic criteria, especially when genetic factors, such as identifying genetically distinct units (e.g., MUs/ESUs), inbreeding, genetic drift, and loss of diversity, are known to have direct impacts on population viability (Laikre 2010; Laikre *et al.* 2020). Existing criteria used by the IUCN ranking system for species of conservation concern was found to be a poor indicator of populations affected negatively by the genetic factors mentioned above (Willoughby *et al.* 2015). In mammals, species categorised to have a declining population predicted decreased genetic diversity, however range size and number of mature individuals were found not to provide a satisfactory indicator. Therefore the current criteria may overlook low genetic diversity, and the viability concerns associated with this, until large census population declines meet the criteria to be recognised as threatened. Willoughby *et al.* (2015) recommended that effective population size and genetic diversity should be considered in conservation rankings. Similarly, Garner *et al.* (2020) recommended three measures where the IUCN and assessors must: i) report method of estimating the number of mature adults, ii) report estimates on traits that allow the estimation of effective population size, and iii) consider effective population size and loss of heterozygosity in assessment criteria.

6.3 Conservation of the Endangered Sanje Mangabey

The overall aim of this thesis was to increase our understanding of the Sanje mangabey so that conservation action plans could be more informed for this relatively understudied Endangered species. The key results of this thesis that should be considered in conservation management are:

i) the Sanje mangabey population is in decline, especially in the Uzungwa Scarp where the group density is significantly lower than Mwanihana *(Chapter Two; Chapter Five)*

ii) the Sanje mangabey is an evolutionary distinct lineage and should be considered at species level, not a subspecies of *C. galeritus* *(Chapter Three)*

iii) the two Sanje mangabey populations are representative distinct lineages and should be considered preliminary evolutionarily significant units *(Chapter Three; Chapter Four)*

iv) the Uzungwa Scarp is threatened by increasing human encroachment and formal protection of this forest needs to increase in line with the effective protection of Mwanihana under National Park regulations *(Chapter Two; Chapter Five)*

v) the Sanje mangabey populations show a high probability of presence and estimated habitat preference in steep montane regions of the forests and therefore active conservation actions for the species should focus on these regions *(Chapter Four; Chapter Five)*

The most unexpected result from the work in this thesis is the level of significant differentiation found between the two Sanje mangabey populations and between the Sanje mangabey and the other *Cercocebus* species (Chapter Three; Chapter Four). This differentiation supports the recommendation in this thesis that each population should be considered preliminary independent evolutionary significant unit (ESU) pending further study. ESUs are most commonly defined as needing to meet two criteria: i) mtDNA haplotypes show reciprocal monophyly, and ii) significant divergence in nuclear DNA allele frequencies (Moritz 1994). The mitochondrial DNA haplotypes showed highly significant genetic differentiation (F_{ST} = 0.95; Chapter Four) and the mitochondrial phylogenetic reconstructions found reciprocal monophyly between the two populations (Chapter Three). The nuclear DNA fragments (TSPY and CD4) used in Chapter Three were originally chosen as highly conserved regions, most suitable for phylogenetic inferences with other *Cercocebus* and Papionin species. Therefore, the nuclear fragments showed negligible differences between the two populations and therefore are likely not to be representative of genome-wide nuclear DNA structure and diversity to infer divergence from. The original plan to generate genome-wide SNPs was not successful and therefore a gap in our understanding remains.

The most comparable study to predict nuclear DNA diversity and structure would be from a study of microsatellite loci in the Udzungwa red colobus (*Procolobus gordonorum*; Ruiz-Lopez *et al.* 2016). This study found significant population structure across five populations isolated to the forest fragments: Uzungwa Scarp, Matundu, Ndundulu, Mwanihana, and Magombera (Ruiz-Lopez *et al.* 2016). Genetic diversity was similar between forest blocks which was attributed to estimated recent gene flow between some forests. The Udzungwa red colobus is far more widespread throughout the Udzungwa Mountains, inhabiting more adjacent forests, and would be estimated to have had more recent gene flow between populations than the Sanje mangabey. Therefore, it would be hypothesised that the population structure of the Sanje mangabey estimated based in nuclear DNA would be similar to the one from colobus and would present greater differentiation due to the distance and isolation of the two populations, with dispersal and gene flow expected to have occurred on a more ancient timescale.

The Sanje mangabey populations were estimated to have diverged 0.77 MYA (95% HPD interval: 0.43 – 1.15 MYA), and the Sanje mangabey lineage to have diverged 2.34 MYA (95% HPD interval: 1.73 – 2.95 MYA) from other *Cercocebus* mangabeys (Chapter Three). The three other *Cercocebus* species previously considered subspecies of *C. galeritus*: the Tana River (*C. galeritus*), agile (*C. agilis*), and golden-bellied (*C. chrysogaster*) mangabeys, had an estimated time to most recent common ancestor

(TMRCA) at 0.56 MYA (95% HPD interval: 0.18 – 0.93 MYA), a time more recent than the TMRCA of the two Sanje mangabey populations. By this measure, the two populations (~100km apart) are more divergent for the four markers used (mtDNA: COII and CR; nuclear: TSPY and CD4), than three species with distributions across central and eastern Africa. Therefore, even with the current absence of nuclear allele frequencies to compare between the two populations, the Sanje mangabey should be considered minimally as two ESUs.

In the latest IUCN Red List assessment of the Sanje mangabey, the species was considered Endangered based on the threat to viability from deforestation and poaching, small area of occupancy and extent of occurrence, and lack of protection in the Uzungwa Scarp (McCabe *et al.* 2019). Considering the findings of the research in this thesis, future assessments and conservation planning must consider an assessment of each population individually as ESUs. The separation of these two populations increases the considered risk of extinction for each by being limited to a smaller extent of occurrence and area of occupancy. This is especially significant for the Uzungwa Scarp where this ESU has very limited protection and therefore at substantial risk of extinction when assessed individually. At the time of the latest assessment for the Sanje mangabey (McCabe *et al.* 2019), no genetic data had been generated for the species and therefore was not a consideration in its assessment. Following the recommendations for inclusion of both genetic diversity and effective population size in ranking criteria (Willoughby *et al.* 2015), each population individually would have a reduced effective population and the overall genetic diversity available to each population would be decreased, perhaps increasing the extinction risk and conservation status at an ESU scale. Based on these considerations, conservation recommendations here will be presented individually for each population as individual ESUs.

For the ESU in Mwanihana, continued protection under National Park status will protect this population, having been proven to be an effective approach over time (Oberosler *et al.* 2020). However, encroachment continues in the forest, most visible in the northern regions of the forest, in the form of snares, poacher camps and timber harvesting (Paddock pers. obs.). The southern region of the forest is within close proximity to National Park Headquarters and therefore has a higher presence of park officials. An increased presence of officials and patrols across the central and northern regions of the forest would aim to deter the illegal encroachment in these regions. Furthermore, with the ongoing development of the main road running alongside the eastern forest edge, the threat of human encroachment is estimated to increase (Cavada *et al.* 2019; Oberosler *et al.* 2020). Even though the Sanje mangabey is estimated to avoid lower elevations and decreases in presence probability with proximity to the forest edge (Rovero *et al.* 2017), the creation of a buffer zone along the forest edge is a recommended conservation action here as this would protect the inner forest and the population from increased encroachment. For the ESU in the Uzungwa Scarp, a significant increase in the active protection of this forest is required to ensure the viability of the resident Sanje mangabey population.

The Uzungwa Scarp has received increased protection since its upgrade to Nature Reserve from Forest Reserve status in 2017, however the difference in protection between the National Park and the Nature Reserve (e.g., one versus 78 permanent staff reported by Hegerl *et al.* 2017) is reflected in the differences in population viability (Chapter Five) and significantly lower group density (Chapter Two). Active patrolling and enforcement increased since the upgrade however this needs to increase more rapidly to slow the rate of impact from human encroachment, aiming to halt the loss of carrying capacity and allow degraded regions to regenerate.

With the protection of each forest, degraded regions of each forest may be able to regenerate and naturally increase the carrying capacity for each population. However, proactive conservation measures would be recommended to accelerate the availability of suitable habitat for the Sanje mangabey. The results of the ENM in Chapters Four and Five highlighted the higher probability of presence for the Sanje mangabey in steep, montane regions and an avoidance of the forest edge and lower elevations. Therefore, we would recommend that native forest is regenerated and expanded in the regions estimated most suitable for the Sanje mangabey. These regions reflect those furthest from human disturbance on the forest edges and would therefore also have an established buffer zone from any estimated future human disturbance. Furthermore, in the last 38,000 years a sediment core from the north-west edge of the National Park estimated montane forests to be becoming increasingly higher in elevation (Mumbi *et al.* 2008). Therefore, ensuring suitable habitat is abundant at higher elevations would potentially protect against decreased carrying capacity from isolation to ever contracting montane forest 'islands'.

Future studies are required to monitor the population size and genetic diversity within each forest to measure the long-term viability of the Sanje mangabey and the efficacy of any conservation approaches used. These studies should be repeated either at regular intervals every few years or as an emergency response to significant stochastic changes in the forest environments. Conservation management plans must be monitored in order to measure the efficiency of current approaches and to learn, adapt and improve in response to the results. Furthermore, continued study of the habituated group in Mwanihana is required to gather long-term life history data that is currently missing for the species. Additionally, the creation and study of more habituated groups across the forest would provide more representative and accurate data. This is also needed across the Uzungwa Scarp as no groups have been studied in this ESU. This population may show differences to Mwanihana that are vital to the understanding of that population's viability and conservation status.

The forests of the Udzungwa Mountains are a conservation priority (Ruiz-Lopez *et al.* 2016; Burgess *et al.* 2007). Protection of these forests ensures that the production of renewable energy from the hydroelectric power stations is maintained, a crucial source of energy previously estimated to be 52.6%

of energy produced in Tanzania (Tanzania Forest Conservation Group 2005). Furthermore, these forests are included within the Eastern Afromontane biodiversity hotspot, with the second highest number of endemic vertebrate taxa out of the world's hotspots (Mittermeier *et al.* 2011), and are considered a primate hotspot (Rovero *et al.* 2015; Barelli *et al.* 2015; Cavada *et al.* 2016) and 'Priority Primate Area' (Davenport *et al.* 2014) with three endemic species: the Sanje mangabey, the Udzungwa red colobus and the recently described kipunji (Rovero and De Luca 2007). The threats described within this thesis to the Sanje mangabey, especially with respect to the low protection of the Uzungwa Scarp, are relevant to these other endangered and endemic species. Therefore, the conservation recommendations made in these studies for increased protection of the forests with priority action placed on the Uzungwa Scarp, will not only benefit the Sanje mangabey but all of the incredible biodiversity across the Udzungwa Mountains.

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