The use of plants by human and non-human primates in altered landscapes: dietary flexibility or local adaptation?

By

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Summary

Non-human primates foraging range and human populations currently overlap extensively in regions of extreme poverty. In these anthropogenic landscapes, people depend heavily on natural resources, sharing space and plants with sympatric non-human primates. To understand the complex social-ecological dynamics of such systems, an integrative approach was applied to evaluate the extent of wild plant overlap between co-existing humans, and western chimpanzees (*Pan troglodytes verus*) and western red colobus (*Pilicolobus badius*) in two national parks in West Africa. Despite being similar in area and demography, Gola Rainforest National Park in Sierra Leone is a semi-deciduous evergreen continuous forest devoid of villages and with low levels of human disturbance, and strong conservation enforcement. Cantanhez National Park in Guinea-Bissau is a mosaic of sub-humid and secondary forests, savanna, mangrove, and agricultural fields and settlements, with little formal protection of the forests and wildlife. High levels of wild plant use by humans were observed in both parks, with people from Cantanhez showing a greater ethnobotanical knowledge. Non-human primate diet across all populations revealed high plant species richness, generally higher in Gola forest. Primate populations at the disturbed site, demonstrated signs of dietary flexibility and consumed a greater number of cultivated items than primates in Gola. Overall, some wild and cultivated plant overlap was detected within each study site, but to a greater extent in the fragmented landscape of Cantanhez national park. Evidence produced in this research, together with local knowledge and expertise, can be incorporated to plan and implement ethical and sustainable conservation strategies and policies, that can conserve threatened primates and their habitat, and respond to people's basic needs.

To mum, dad and sis

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Abbreviations and Acronyms

CNP	Cantanhez National Park
CSSL	Conservation Society of Sierra Leone
DNA	Deoxyribonucleic Acid
GRNP	Gola Rainforest National Park
HDPE	High Density Polyethylene
HTS	High-throughput Sequencing
IBAP	Instituto da Biodiversidade e das Áreas Protegidas
ITS2	Second Internal Transcriber Spacer
LRT	Likelihood Ratio Tests
MGLM	Multivariate generalized linear model
NMDS	Non-metric multidimensional scaling
NP	National Park
NTFP	Non-Timber Forest Product
PA	Protected Area
PCR	Polymerase Chain Reaction
RSPB	Royal Society for the Protection of Birds
SP	Super-pool

IUCN Red List Conservation Status

Three threatened categories (Mace and Lande 1991)

CR	Critical	50% probability of extinction within 5 years or 2 generations, whichever is longer.
EN	Endangered	20% probability of extinction within 20 years or 10 generations, whichever is longer
VU	Vulnerable	10% probability of extinction within 100 years.

Study species

EN	*Piliocolobus badius*	Suspected to have undergone a decline exceeding 50% during the past three generations (ca 30 years) caused by human-induced habitat loss and hunting.
CR	*Pan troglodytes verus*	Estimated to have experienced a significant population reduction in the past 50 years, and it is suspected that this reduction will persist in coming years. This is mostly due to high levels of poaching, and loss of habitat and habitat fragmentation resulting from human activities.

CHAPTER 1. General Introduction

Gola Rainforest National Park, Sierra Leone.

1.1 Research background

Human population growth implies expanding anthropogenic areas and reducing forested landscapes, which in turn increases proximity with wildlife (Woodroffe et al. 2005; König et al. 2020). This is especially evident for non-human primates, whose ranges overlap extensively with human populations characterised by high levels of poverty (Estrada et al. 2017; Bloomfield et al. 2020). Obtaining a better understanding of how co-existing species utilise the same space and resources is vital for conservation projects which aim to apply achievable and successful strategies to protect biodiversity and ecosystems. One area of interest is the sharing of wild plants, as humans and wildlife use wild plants for food, tools, and shelter, among other uses.

Despite being protected areas, national parks (NPs) are far from free of deforestation, hunting and other destructive activities that put biodiversity at risk, and are a potential for human conflict as many impose access restrictions to local human populations (Makombe et al. 1993; Madden 2004). Moreover, different NPs have different characteristics that contribute to variation in human-wildlife-nature relationships. Therefore, it is important to perform site-specific studies, that cover several elements of the ecosystem, including humans, and integrate different disciplines that can address some of the variables of the social-ecological system. Although distinct, comparing study sites can provide a broad view and new insight into how humans and animals adapt to more disturbed landscapes, and how they interact and co-exist in a type of environment that is tendentially increasing, those of anthropogenic habitats. Applying an interdisciplinary approach to study social-ecological systems has its challenges, such as aligning different types of data, communicating across disciplines, and including social sciences data in conservation plans, but it has been increasingly recognised as an integral requirement for conservation success (Lischka et al. 2018; Hockings et al. 2020). This research uses two NPs with similar demographic and economic characteristics, located in West Africa, as study sites to explore the co-use of wild resources by humans and non-human primates. Co-use will be evaluated across distinct vegetation covers (continuous vs fragmented), human presence and pressure (low vs high) and conservation enforcement (high vs low). Ultimately, by applying an integrative approach to study such complex interactions and ecosystems, solid evidence together with local knowledge can be put together to plan and implement ethical and sustainable conservation strategies and policies that can protected biodiversity and consider people's basic needs.

1.2 Human and non-human primate co-existence

Humans and other primates have shared ecosystems for millennia, sometimes in a beneficial way, and other times in more negative and conflicted manner (Tutin and Oslisly 1995). Throughout this thesis, references to co-existence reflect this paradigm of simultaneous space and resource use. Sympatric organisms modify their own niches as well as those of other species, through their activities and choices (Odling-Smee et al. 2013). Non-human primates (hereafter primates) have an important ecological role in the ecosystem, as they contribute to forest regeneration as seed dispersers (Chapman and Dunham 2018), potential pollinators, seed predators (Struhsaker 2010) and improve the soil conditions for plant germination with excrement deposition (Kalbitzer et al. 2019). Humans on the other hand, can extract or consume naturally occurring plant species creating opportunities for new plant growth, and amend and manage resources to maintain its stability and increase its productivity (Bharucha and Pretty 2015).

Living in close proximity with primates means having some native vegetation area that can provide a good source of plants and animals to humans, but also ecosystem services such as soil retention and fertility, and water conservation (Daily 1997; Costanza et al. 2017). In addition, primates in many areas of Central and west Africa, Brazilian Amazonia, and Southeast Asia are an important source of protein and income to humans (Refisch and Koné 2005), although health issues associated with primate meat consumption are a major concern (Devaux et al. 2019). However, one of the costs linked to having primates as neighbours, is crop damage (Hill 2005). For primates, plantations can be advantageous as they may provide the required nutrients during periods of low food availability in the wild, but crop feeding can also pose a risk to primate survival (Naughton-Treves et al. 1998; Hockings and McLennan 2012). Generally, the costs for primates living close to humans outweigh the benefits. Hunting for bushmeat and the pet trade, and transmission of parasites and diseases increase, and natural resources may decrease in more anthropogenic habitats (Goldberg et al. 2008; Estrada et al. 2012).

From a conservation point of view, natural resource exploitation by humans would be sustainable, but in times of globalisation, and environmental and economic uncertainty, this is not the case in many situations. Once-pristine natural ecosystems are rapidly disappearing or being altered into a human-modified mosaic landscape (Tscharntke et al. 2012), and biodiversity is being lost at unprecedented rates (Ceballos et al. 2015). These declines are a consequence of increasing anthropogenic activities such as land clearance for agriculture and infrastructure construction, unsustainable use of natural resources, bushmeat hunting and the pet trade, and ultimately climate change (IUCN, 2017). Spatial and ecological overlap between humans and primates is increasing rapidly, and demands for natural resources can decrease supplies or alter forested habitats, forcing primates to adapt or move to other locations(Marsh et al. 2013).

Approximately 60% of primate species are at the verge of extinction in most of their habitat (Estrada et al. 2017) and for many populations, their only safety are protected areas, such as National Parks (NP). Protected areas are estimated to cover 15% of the planet and can act as a refuge for many species (UNEP-WCMC, & IUCN 2022). However, these areas can be a potential for conflict between stakeholders, as many NPs restrict local human populations from accessing the land and its resources, and at the same time wildlife numbers increase due to successful conservation, increasing the chances of human-wildlife encounters and potentially crop feeding, exposing both groups to higher and more frequent risks (Makombe et al. 1993; Madden 2004; Mackenzie 2012). In order to choose the best management practices to apply in a protected area that will minimise human-wildlife encounters and guarantee biodiversity conservation, it is critical to understand the needs of all elements of the dynamic ecosystem (Milich 2017). Furthermore, more research needs to focus on a specific habitat that many threatened primate species currently use and/or live in, that of agroecosystems. This type of habitat covers one quarter of the land surface (ca. 50 million km^2) and is as diverse as a simplified pastureland, or a complex polyculture or agroforestry systems (Estrada et al. 2012). Deforestation was estimated at 10 million hectares per year between 2015 and 2020, having reduced the area of primary forest worldwide by over 80 million hectares since 1990 (FAO 2020). Land conversion to agriculture in tropical regions has caused major forest loss, habitat degradation and fragmentation, and has had an impact on the distribution of primates across the landscape (Donald 2004; Estrada et al. 2017). Understanding how primates respond to seasonal and annual variation in food availability in a natural and continuous forest, and how they adapt to harsher resource limitations in anthropogenic landscapes, is important for primate and natural resource conservation, ultimately contributing to the wellbeing of rural communities.

1.3 The importance of wild plants for people, red colobus and chimpanzees

An estimated 880 million people worldwide depend on wild plants to provide food security, especially in times of environmental and economic uncertainty (FAO 2020). In West Africa, particularly in the Upper Guinean Forest hotspot, most countries' political situation is unstable. The rapidly growing human population in rural areas live on less than $1-1.25 per day and their existence relies almost entirely on natural resources, particularly non-timber forest products (NTFPs) (UNPD 2017; Rural Poverty Portal 2017). Wild plants are a substantial part in the diet of humans, provide material for house, tool and boat construction, contribute to the economy of local communities, constitute an important asset in cultural and social events, and medicinal plants play a crucial role in community health (FAO 2001; Belcher et al. 2005; Bharucha and Pretty 2015; Hwang et al. 2020).

Primates use trees as shelter, places of socialisation and as a means of daily travel and dispersal. Equally important, fruit, leaves, and seeds produced by trees and other plants are the most common food items consumed by primates (Harding 1981; Oftedal 1991; Hohmann et al. 2012). The quality and quantity of food items consumed can determine growth and reproductive success, body size, gut morphology, and behavioural and social composition (Campbell et al. 1999; Marshall and Wrangham 2007; Gussekloo 2008). Resource availability can influence species density and group size, habitat utilisation and activity patterns, among others. For example, in times of low food availability, chimpanzee communities in Tai NP, Côte d'Ivoire, have shorter daily travel distances. When ripe fruit is highly available, their territory expands presumably because time can be invested in territorial defence (Herbinger et al. 2001). These two groups are highly dependent on forests and their products, and given that primates and human populations co-exist and their habitats overlap (Estrada et al. 2017), it is expected that a portion of forest resources is co-utilised.

1.4 Primate flexibility to food availability in natural and altered habitats

Contrary to what was proposed in early work on life-history evolution by Dobzhansky 1950, tropical habitats in which most primates live are not climatically "constant" (Jones 2011). In fact, ecological studies in the tropics suggest a clear seasonal variation with changes in rainfall, temperature, photoperiod and phenology (van Schaik et al. 1993; van Schaik and Brockman 2005; van Schaik and Pfannes 2005). Exposure to extreme environmental variation raises the possibility that primates are generally adapted to spatial and temporal variation in food resources (Ma et al. 2017). Tree dieback due to cohort senescence, and alterations in the vegetation composition resulting from natural disasters or heavy natural herbivore grazing, can have a negative impact on some primate populations, but others can thrive in subsequent forest regeneration events (Isabirye-Basuta and Lwanga 2008). However, when such changes occur at a faster rate and more frequently, a characteristic of the Anthropocene, primates must adapt to the new environments to survive.

Distinct levels of habitat disturbance suggest variation in the level of flexibility for different species and populations, as some primates may not have the required capacity to adapt to such changes (Onderdonk and Chapman 2000; Isabirye-Basuta and Lwanga 2008; Struhsaker 2010; Dunham 2017). In the past 20 years, empirical evidence on primate behavioural flexibility and adaptation to forest fragmentation and anthropogenic habitat, has been growing (Henle et al. 2004). This field of research shows that not all species decline towards extinction, and some have higher resilience than expected. Primates exhibit flexibility to the environment in various forms, but dietary adjustments are the most frequently reported in the literature (McLennan et al. 2017).

When faced with natural or human-derived fluctuations in food availability, primates will respond according to their biological and ecological characteristics. Some primates may shift their diets, such as the blue monkey (*Cercopithecus mitis*) groups living in a logged forest that showed higher fruit consumption and more consistent diet than a group in the unlogged forest (Fairgrieve and Muhumuza 2003). Some apes and monkeys complement their diets with cultivated items which are palatable, energy-rich and easily digestible (Naughton-Treves et al. 1998; Hockings and McLennan 2012). They can even change their ranging and social patterns, for example a group of Tonkean macaques (*Macaca tonkeana*) living in a heavily altered habitat that exhibited a greater home range, foraged for longer in microhabitats within their range, and spent more time on the ground than a group inhabiting a minimally disturbed territory (Riley 2008). Activity budgets can be adjusted in time spent feeding, resting, travelling and in social behaviour, as was the case for a red colobus group (*Piliocolobus kirkii*) living in a habitat rich in herbaceous plants. This low-quality plant type represented a large proportion of their diet, especially when tree foods were scarce, and individuals spent less time moving, and more time grooming and in aggression activities, than the forest group where tree foods were more available (Siex 2003). Intra and inter-specific competition for food can also vary due to food availability. For example, among three different monkey species, competition for the most common item (e.g. fruit) was higher when food availability was also higher and diets less diverse. During periods when fruit production was recovering or decreasing, primates had the most distinct diet having lower levels of dietary overlap and consequent competition events (Guillotin et al. 1994).

Despite their apparent dietary and behavioural flexibility, primates are highly vulnerable to tropical forest loss and degradation because they are arboreal, forest-dependent, and sensitive to ecosystem changes (Isaac and Cowlishaw 2004). A global meta-analysis quantifying the effects of anthropogenic habitat modification on primate community determined that human-induced habitat change had a negative effect on primate assemblages, and could potentially lead to drastic population declines (Almeida-Rocha et al. 2017). The extent to which primates are able to survive alongside people and in degraded environments in the long term is yet to be understood. Recently, populations of South American primates that appeared to sustain adaptive potential and resilience in their reduced and fragmented habitat, were shown to experience limitations at the population level. The high individual behavioural and dietary flexibility of howler monkeys (*Allouata* spp.) in a range of shrinking habitats masked the limited capacity of the population to recover from discrete disturbances, as was the case when recent yellow fever outbreaks resulted in several local extinctions (Bicca-Marques et al. 2020). These and other factors operating at the ecological and biological levels (e.g. restricted gene flow due to isolated population in small fragmented pockets of forest, reduce genetic diversity and increase species vulnerability to stochastic events, such as natural disasters and disease) can determine the capacity of primates to survive in non-natural environments.

1.5 Research in complex social-ecological systems

In landscapes where humans and primates co-exist, it is crucial to assess the human and cultural characteristics of the ecosystem, as well as the ecological and biological patterns of primate populations to develop effective strategies for primate conservation and human wellbeing (Fuentes and Hockings 2010). Research into these dynamic systems is only possible when a combination of social and biological science methods are applied and interlinked (Newing 2011; Fuentes 2012; Dore et al. 2017; Setchell et al. 2017). However, interdisciplinary studies can be presented with barriers in discipline-specific culture, research methods, terminology, and in integrating a bidirectional approach of interactions between people and wild animals due to the difficulty of some researchers considering humans an integral part of the ecosystem and not separate from nature (Parathian et al. 2018). Efforts have been made in the field of Ethnoprimatology, which combines social and biological science approaches and includes humans in the system, to offer a more holistic understanding and perspective in primate research (e.g. Fuentes 2012; Riley 2013; Malone et al. 2014; Remis and Jost Robinson 2017). This inter-disciplinary research brings knowledge that could not be achieved with a single disciplinary approach, but to obtain a complete picture of the system, political, cultural, socioeconomic and ethical elements need to be incorporated (Hill 2002; Nekaris et al. 2010; Whitty 2018).

1.5.1 Mixed methods to explore wild plant overlap

To understand the extent to which humans and primates use the same wild plants in one ecosystem, it is necessary to apply an inter-disciplinary approach. In primatology, feeding ecology studies focus on food acquisition and processing, habitat utilisation, foraging strategies, ecology and sociality (Robbins and Hohmann 2006; McKinney and Dore 2018), but usually exclude any interaction with human foods (e.g. crops) or wild plants exploited by humans as these are not seen as natural food items. Similarly, in Ethnoprimatology a wide range of topics are covered, such as people's perceptions on primates and primate behavioural flexibility to disturbed environments, but few focus on the overlap of resources by humans and primates, though the incorporation of human food in primate diet is one of the most commonly documented phenomena (McLennan et al. 2017). The reason for such a limited number of studies examining the overlap of natural resources between humans and primates may be explained by barriers to cross disciplinary research previously described. Furthermore, the botanical knowledge needed to collect correct taxonomic information on the wide range of plants used by humans and primates may discourage many scientists from using a cross-disciplinary approach (Hockings et al. 2020). As a result, most studies focus on the co-use of one important plant species for both groups (Kinnaird 1992; Reynolds et al. 2012; Sheil et al. 2012; Ramon 2017)

Two studies have explored the co-utilisation of all or one type of wild plant (e.g. fruit) by chimpanzees and humans in West Africa (Bossou, Côte d'Ivoire, Sugiyama and Koman 1992; Cantanhez NP, Guinea-Bissau, Hockings et al. 2020), and one study in Lore Lindu NP, Sulawesi, Indonesia evaluated the overlap between Tonkean macaques and humans (Riley 2007). Social data were obtained using formal or informal interviews conducted with people known to harvest wild resources in the same areas of the primate in question. Ecological data included a combination of meteorological records, phenology data or plant species identification in the area. The latter has been achieved with the help of botanists or local plant experts. All three studies were performed at a fine spatial scale, focusing on one location, addressing informants in a few villages (max. 3), and collecting data on the diet of one primate species only. Biological data were collected through direct observation of food consumption, feeding traces encountered in the area, or examination of plant parts in faeces. In Bossou, plant lists for both groups were gathered, and plant species co-use identified. In Cantanhez NP, scores for the human use value of each fruit plant and for the quantity of fruit consumed by chimpanzees were compared and analysed against phenological data. In Sulawesi, the salience score of each item (how much knowledge and how important the plant is to the informant) was calculated based on the information obtained through a freelisting technique, which asked respondents to list all forest resources and tree species known to them. The salience score was compared with the list of plants in the primates' diet and plants used by both were identified. This study included other techniques and analysis to assess crop feeding by macaques in cocoa plantations.

1.5.2 A more modern approach to assess diet: DNA-metabarcoding

Analysis of plant and animal items in the diet of primates has traditionally required direct observations of feeding behaviour, and/or macro- or microscopic identification of digested food remains from stomach content or faecal samples. These studies provide crucial information on dietary composition, rates of consumption, preferred items and plant parts consumed, as well as specific places and times when food was ingested (Hohmann et al. 2012). In some cases, novel behaviour has been reported, such as primate meat hunting by chimpanzees (Boesch and Boesch 1989) or nut-cracking of hard shells with stones (Carvalho 2011). However, to obtain data in the field, these methodologies require long periods of observation, long distances travelled to follow the groups, and habituated primates are preferable (Doran-Sheehy et al. 2006). Additionally, the quality of the data often depends on the researchers expertise, and the type of habitat inhabited by the focal primate (e.g. it is harder to observe feeding events in higher and denser canopy) (Struhsaker 2010). Furthermore, taxonomic identification of the food items after gut passage is challenging, as highly digested plant remains in faeces can be indistinguishable.

A relatively recent molecular approach using DNA sequences from species ingested by primates has the potential to provide a more accurate and exhaustive analysis of the diet composition and variation (Pompanon et al. 2012; Taberlet et al. 2012). DNA metabarcoding allows for the rapid identification of multiple taxa in a single experiment by combining DNA taxonomy and high-throughput sequencing (Coissac et al. 2012; Pompanon et al. 2012; Ji et al. 2013). By amplifying one or more targeted DNA sequences that exhibit consistent differences between taxa and are highly variable, it is possible to identify plants and animals against taxonomic reference databases of the organisms in question. DNA from several organisms can be extracted from non-invasive samples, such as faeces, as well as soil, water and air samples

(Valentini et al. 2008; Clare et al. 2021). Nuclear or chloroplast DNA are amplified for plants, and for animals, nuclear or mitochondrial DNA segments are sequenced (Symondson 2002). These techniques provide an opportunity for researchers to obtain unprecedented information of the diet, parasitology, gut microbiota and population genetics of elusive and threatened species, such as primates (e.g. Quéméré et al. 2013; Wilcox and Hollocher 2018; Gogarten et al. 2020; Milner et al. 2021). Thus far, to my knowledge six published studies have used a DNA metabarcoding technique to explore primate diet, but only three have used plant-specific primers. The first study used this approach to reveal dietary diversity and plasticity in 96 lemurs and was performed using the chloroplast *trn*L universal plant marker (Quéméré et al. 2013). *Propithecus tatersalli* in north-eastern Madagascar fed on 130 plant species including naturalised and cultivated items and showed a high flexibility of foraging strategies towards degraded habitat. More recently, Osman et al. (2020) used the *trn*L plant marker to describe 145 plant species in the diet of three extremely elusive *Macaca arctoides*, residing only in Pertis State Park in Peninsular Malaysia. Another study analysed data from *Cebus capucins* from a field station in Costa Rica to compare the efficiency of methodologies (direct feeding observations and DNA metabarcoding) and chloroplast plant markers (*rbc*L and *trn*L) in 170 faecal samples (Osman et al. 2020). The *trn*L maker was determined to be more robust for plant dietary analysis, identifying 69 species of plants in the capuchin's diet.

1.6 Cross-disciplinary research aiding primate conservation

Primate responses to habitat disturbances can usually be associated with particular alterations, such as elimination of important food trees (e.g. selective tree felling) or density increases in new food sources (e.g. invasive or cultivated species) (Johns and Skorupa 1987). Hence, gathering spatial and temporal data on the diet of primate populations living in natural and altered habitats where the floristic composition comprises of primary and secondary forest plants, invasive plants and crop species is crucial to aid conservation programmes and understand how non-human primates survive in such landscapes (Johns and Skorupa 1987; McLennan et al. 2017;

Struhsaker 2017). Yet, primate feeding ecology benefits from a broad perspective of the subject. Addressing research on population and community processes is as important as focusing on the nutritional composition of plants and digestive abilities of primates (Conklin-Brittain et al. 1998; Ryan et al. 2013). Moreover, gaining insight into the role of primates as seed dispersers, predators and prey, competitors, etc., may contribute to the conservation of the ecosystem that supports primates and human communities (Robbins and Hohmann 2006).

With humans being major niche constructors and altering the habitat of other primates (Fuentes 2010) but equally being natural resource consumers, it is essential to integrate social sciences with biological science approaches to understand which plants are selected by which party and why, inside and outside protected areas, and the extent of sustainable wild plant co-use (Parathian et al. 2018). This information can identify trees and other plants that play a significant role in primate survival and human wellbeing, and develop tree protection and reforestation plans that maintain forest cover, increase connectivity and ecological resilience (Hockings et al. 2020). Dietary data and the economic value of crops can also clarify the real impact of primate crop feeding, and create mitigation measures to reduce or prevent people’s animosity towards wildlife, that may arise from resource overlap (Riley 2007; Wallace and Hill 2012).

Plant co-use does not directly imply competition for resources, and although many of the studies on primate flexibility in anthropogenic habitats include information on negative interactions between humans and primates, research should also include positive and neutral events (McLennan et al. 2017). Consequently, a cost-benefit approach should be applied in interdisciplinary research and conservation to identify appropriate mechanisms that maintain sustainable human-wildlife co-existence. Research on the co-utilization of wild plants by sympatric species, including humans, in complex social-ecological systems, may, in the near future, contribute to the development and implementation of evidence-based habitat and species conservation strategies and policies, and the success of ecosystem management (Mace 2014; Parathian et al. 2018; IUCN SSC 2020; Linder et al. 2021).

1.7 Apes and monkeys of west Africa

Western red colobus (*Piliocolobus badius*) and western chimpanzee (*Pan troglodytes verus*) are examples of some of the world's most threatened mammals, with most populations surviving in small and isolated habitat fragments (Oates and Nash 2011). In this thesis, the diet of the colobine, a folivore, and the chimpanzee, a frugivore, will be investigated using a molecular approach (DNA metabarcoding). The two primates have similar geographic distributions (Fig. 3.1 in Chapter 3, and Fig. 4.2 in Chapter 4) but distinct dietary requirements, forest dependency and adaptability to human-disturbed habitats. Populations of western red colobus and chimpanzees are found living in sympatry in the two national parks of this study. Previous studies have detected dietary flexibility when exposed to food shortage, including consumption of cultivated items. However, as red colobus spend most of their lives in the tree canopy, they are likely more vulnerable to habitat degradation and deforestation than chimpanzees. Below is a short description of each primate, but Chapters 3 (section 3.3.2) and 4 (section 4.3.2) provide a thorough account of the western chimpanzee and red colobus, with specific information on populations living in Gola Rainforest NP in Sierra Leone, and Cantanhez NP in Guinea-Bissau.

1.7.1 The western chimpanzee

The western chimpanzee is one of the four subspecies of *Pan troglodytes*, the most widely distributed of all African apes (Butynski 2003; Oates and Nash 2011). The habitat occupied by this species is in the African forest belt and is very diverse, ranging from dense rainforests to savannas and farmlands (Davies 1987; Hohmann et al. 2012). Much of their habitat is occupied by humans who increasingly deforest areas for the implementation of agriculture, posing a danger to the species. In Sierra Leone, a population survey registered a higher number of chimpanzees in non-protected areas (c. 3100 ind.) than in forest reserves (1460 ind.) (Brncic et al. 2010).

Western chimpanzees are typically frugivorous, preferring high-quality food and feeding on rare tree species, than more commonly available ones (Wrangham et al. 1998). This preference limits the species diversity of their food, but a higher number of different plant species will have a larger contribution in their diet (Wrangham 1977). Chimpanzees also include other food items in their diet, such as invertebrates, small mammals and even other primates like as red colobus (Boesch and Boesch 1989). In anthropogenic environments, this dietary requirement (fruit) can trigger crop foraging in search of fruits cultivated by humans (Hockings and McLennan 2012; Hockings and Sousa 2013; McLennan and Ganzhorn 2017). This behaviour increases encounters with humans that can result in hostile human-wildlife interactions, disease transmission, and increased hunting or capture for the pet trade (IUCN SSC 2020). Consequently, all four subspecies are under the IUCN Red List category (2022) Endangered (EN), with *Pan troglodytes verus* (one of the two focal species of this thesis) being classified as Critically Endangered (CR) with a declining population trend (Humle et al. 2016).

1.7.2 The western red colobus

Colobinae is a subfamily of the Old-World monkey family (species of African and Asia) which includes the genus *Piliocolobus* (red colobus). Colobines have a complex evolutionary history, resulting in distinct anatomy, ecology and social dynamics (Ting 2008; Linder et al. 2021). This group has always been regarded as folivorous, but research has shown that seeds are their most important food item, and these primates only turn to leaves during times when other preferred food is in short supply (Koenig and Borries 2001). Their diet is more generalised than other primates because they have a lower-quality diet feeding on more commonly available plant species (Kaplin et al. 1998; Doran-Sheehy et al. 2006). In a natural and healthy environment, colobus monkeys are known to include a wide diversity of plants in their diet and feed more often on fewer items (Chapman and Chapman 1999; Struhsaker 2010).

Even though more studies have focused on these monkeys, most species are still understudied (Davies and Oates 1994) and the current conservation status of certain species may change if further investigation is carried out (Colobines are classified as Vulnerable, Endangered and Critically Endangered, IUCN 2022). Red colobus are found in sub-Saharan Africa but no further south than Tanzania, with a patchy distribution and mostly absent in equatorial Africa (Struhsaker 2010). The western red colobus is one of 17 species of the genus *Pilicolobus*, and it is the only one that subdivides into two subspecies. Temminck's red colobus populations (*P. b. temminckii*) which occur in small fragments of declining vegetation, such as mangrove, gallery forests, savanna and sub-humid forests, from The Gambia and southwest Senegal to western Côte d'Ivoire, including the territory of Cantanhez NP in Guinea-Bissau. The other form, the Bay red colobus (*P. b. badius*), is most abundant in undisturbed forests with three of the five main populations in Sierra Leone: Gola RNP, Tiwai Island Wildlife Sanctuary and Outamba-Kilimbi NP (Linder et al. 2021).

1.8 Study sites

Fieldwork was conducted in two national parks (NP) in West Africa, under long-term research projects implemented by my supervisors. Gola Rainforest National Park (RNP) in Sierra Leone, and Cantanhez National Park (NP) in Guinea-Bissau have been officially recognised as NPs for over 10 years. The parks lie in the African wet tropical climate zone (Fig. 1.1) and protect some of the most biodiverse tropical forests in West Africa (White 1983; Myers et al. 2000). Both areas are subjected to high levels of rainfall, with peak precipitation in July and August, and the driest period from December to April. However, these ecosystems are increasingly threatened with anthropogenic pressures due to rapid demographic and economic growth, including deforestation, over-exploitation of natural resources, and hunting (World Bank 2020; UNPD 2022).

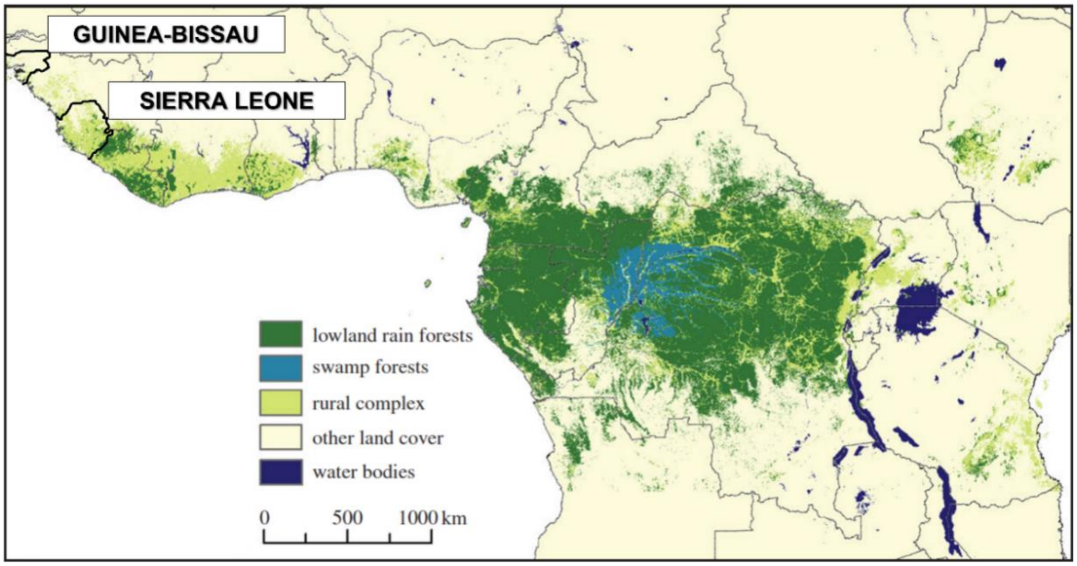


Figure 1.1 Rainforests distribution in continental Africa. The map indicates the lowland rainforest in Gola RNP, Sierra Leone, and the rural complex habitat in Cantanhez NP, Guinea-Bissau. Image modified from a map derived from MODIS data (Mayaux et al. 2013).

The recent history of both countries follows a similar path of colonisation, independence, and economic and governance instability. Guinea-Bissau and Sierra Leone are two of the countries with the lowest income worldwide ($1.9 per day per capita), with severe famine levels and poor health services (UNIOGBIS 2017; Robinson 2019; World Bank 2020). The land where the parks are located are home to several community groups with different cultures and traditions (Leach 1994; Temudo 2009). For example, in Cantanhez NP, land tenure is regulated by the Nalú community group (Frazão-Moreira 2009), and in Gola RNP by Mende families (Tubbs 2015). Rural livelihoods are heavily dependent on forest resources, such as non-timber forest products (NTFPs) (FAO 2013; Meinhold and Darr 2019). In recent years, the Parks' management teams have improved their conservation tourism as an additional and alternative income for local populations (Tubbs 2015; Catarino and Palminha 2018). Research has developed in both parks for a couple of decades, albeit interrupted during civil war years (1991-2002) in Sierra Leone. Additionally, national and international charities have led programs to improve and develop the economy, health and education of local communities.

Research permits

Formal permission to conduct fieldwork involving non-invasive biological sample collection and interviews with local residents, was obtained for Gola RNP from the Research and Monitoring Department of the Gola RNP in Kenema, Sierra Leone, and for Cantanhez NP from Instituto da Biodiversidade e Áreas Protegidas (IBAP) in Bissau, Guinea-Bissau. Research permission was also obtained from the town chiefs from the villages close to or in the forests surveyed, and the villages interviewed. For the social sciences component of the research, study and interview design were approved by the ethics committee of the Centre for Research in Anthropology (CRIA) in Portugal, and BIOSI Ethics at Cardiff University, U.K.. The investigation complies with The European Code of Conduct for Research Integrity.

1.8.1 Gola Rainforest National Park – Sierra Leone

1.8.1.1 The Republic of Sierra Leone

Sierra Leone is a coastal country of West Africa (71,740 km^2) bordered in the north and east by the Republic of Guinea, in the southeast by Liberia, and the Atlantic Ocean to the west and southwest (Fig. 1.2 a)). The latest population census recorded over 7 million inhabitants, belonging to 18 sociolinguistic groups, with the most prevalent being Temne in the northwest, and Mende in the southeast (SSL 2015). Freetown is the capital, and Kenema the third largest city in the country and the closest to Gola RNP (30 km). The civil war lasted between 1991 and 2002, was initiated around the Gola Forest, and caused changes to the land cover due to population redistribution (Burgess et al. 2015). In 2014 the same region was severely impacted by an Ebola outbreak (Robinson 2019). Agriculture is a predominant activity in the country, occupying approximately 55% of the total land area, while forest cover is at an estimated 36% (FAO 2016). Sierra Leone is in a transition zone between continually wet tropical rainforest and a tropical savanna climate (Gabler et al. 2008), and rich in natural resources including valuable minerals such as diamonds and gold. Although there are seven NPs and several other protected areas in Sierra

Leone, deforestation is a serious threat to floral and faunal biodiversity. Deforestation clears 30,000 ha per year although this may be underestimated as it used data from 1975-1986 and does not consider population and economic growth since then, nor the impact of forest occupation by rebels during the civil war (Wadsworth and Lebbie 2019).

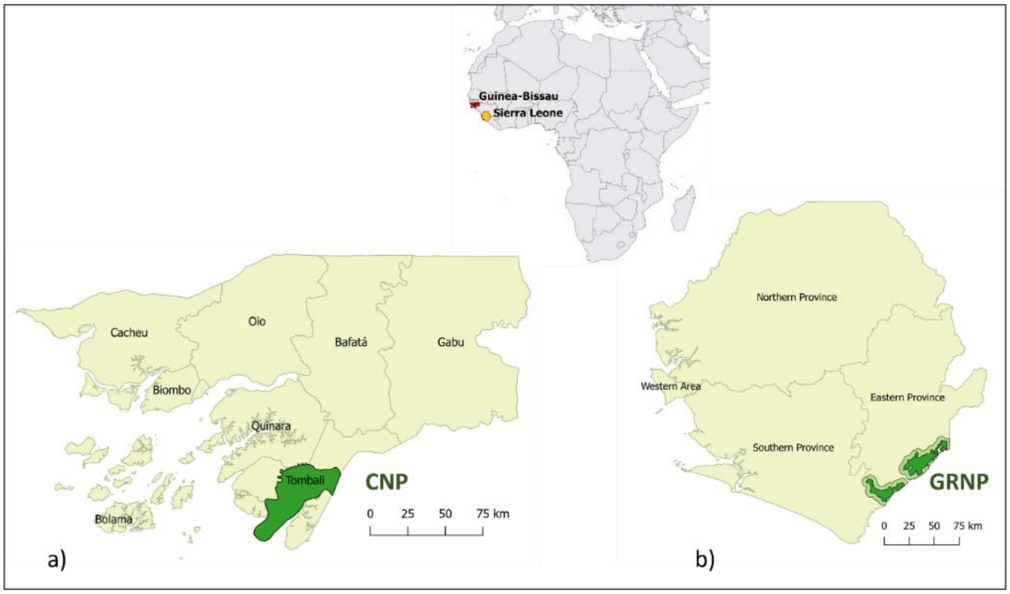


Figure 1.2 Location of the two national parks in West African countries. Cantanhez National Park (NP) on the left is approximately 1,000 km^2 of a mosaic of subhumid forest, secondary forest, mangrove, savanna, human settlements, and agricultural fields, located in the south of Guinea-Bissau. Gola Rainforest National Park (RNP) on the right, is a continuous semi-deciduous area of ~750 km^2 in southeast Sierra Leone.

1.8.1.2 Geography, climate, biodiversity and threats

The limits of Gola RNP (07°18'2"N and 07°51'00"N, and 11°21'13"W and 10°37'40"W) were established at the end of the 19th century as a game reserve, and later as a logging and mineral extraction area. Only recently it has obtained the designation of National Park (in 2011) by the Government of Sierra Leone. The protected area covers ~750 km^2 of tropical rainforest in the southeast of the country and is formed of three forest blocks: Gola North, Gola Central and Gola South (Fig. 1.3). The southeast boundary of the Park adjoins two Liberian protected areas, the Gola Forest National Park and the GolaMA project community forest. Gola RNP does not contain any human settlements and most forest exploiting activities are banned, but sustainable harvest of NTFPs and fishing are permitted and directly benefit forest edge communities (FECs) (Tubbs 2015). Gola RNP is a catchment area for rivers Moro, Mahoi, Mano and Moa (Barca et al. 2018a), and an important water supply for villages in the surrounding area and beyond. Immediately adjacent to the project area, and truncated by the Sierra Leone-Liberian border, is a 4 km wide buffer zone (also known as community forest) of forested and non-forested area defined as part of a REDD+ project. This UN programme aims at mitigating climate change by Reducing Emissions from Deforestation and Forest Degradation in Developing Countries (REDD), plus sustainably manage forests, and conserve and enhance forest carbon stock. In this region, approximately 122 FECs can undertake most activities, although hunting for protected species is illegal (Tubbs 2015). The territory beyond the buffer zone extends to the boundaries of the seven Chiefdoms where Gola RNP is located and includes 373 settlements.

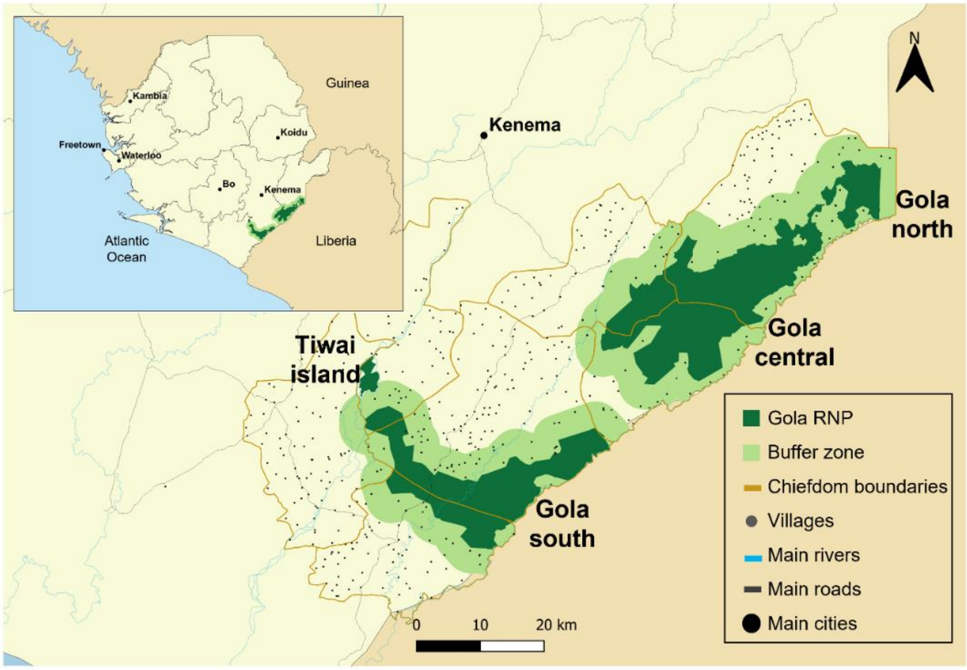


Figure 1.3 Gola Rainforest National Park map. Depicts the protected forest in dark green, and the community forest (buffer zone) where 122 villages (Forest Edge Communities – FEC) are located. Adjacent to the park, is the 12 km^2 Tiwai Island Wildlife Sanctuary, a community conservation initiative established in 1987.

The reserve is a vast area of intact dense lowland moist evergreen and semi-deciduous forest, strongly managed for forest conservation and with a clearly demarcated boundary (Lindsell and Klop 2013). Gola forest is dominated by trees reaching 40m in height in the primary forest, large areas of secondary forest resultant from historical logging activities prior to the civil war and former farming sites, and forest and herbaceous swamps (Klop et al. 2008; Brncic et al. 2010). Intensive logging has taken place in Gola South block and the western part of Gola Central. The latest botanical survey in Gola RNP identified 899 plant species, of which 232 were trees (Klop et al. 2008). The most abundant plant families are Fabaceae and Euphorbiaceae, with *Heritiera utilis*, *Brachystegia leonensis* and *Cynometra leonensis* the most common trees (detailed information on forest types and common plants can be found in Table A.1.1, in Appendix 1). The first two are under the Vulnerable conservation category (IUCN 2022) mostly due to logging and wood harvesting, and the third is Near Threatened for the same reason. All three are used as NTFPs for construction and food (*PROTA4U* 2022). The rainy season starts in May and ends in November, with July and August being the hottest months. Young foliage production peaks at the beginning of each season, with flowering occurring only in the early dry season and followed by the fruiting season. Despite there being two foliage peaks, young leaves are present throughout most of the year due to differences in leaf production from different plant species (Oates 1988).

Research in the park has been conducted for over 20 years and high biodiversity has been confirmed by the presence of 47 large mammal and 330 bird species (Klop et al. 2008), over 500 butterflies (Belcastro and Larsen 2006), 41 bats (Weber and Fahr 2009) and 31 fish (Payne et al. 2009). Many of these species are endemic and listed as threatened, including the Diana monkey (*Cercopithecus diana diana*), western red colobus (*Piliocolobus badius badius*) and King colobus (*Procolobus polykomos*), and western chimpanzee (*Pan troglodytes verus*) (Lindsell et al. 2011). Gola RNP is one of the largest remaining forests of the Upper Guinean biodiversity hotspot, an especially important priority area for the conservation of the world’s primates (Myers et al. 2000; Darwall et al. 2015). Deforestation is not a concern in Gola RNP (Wadsworth and Lebbie 2019), but unsustainable use of NTFPs and hunting may be a threat to plant and animal species. The latest research on primate hunting in Gola RNP suggests high hunting intensity, being higher in the community forest, though the counteracting effect of ground patrolling was not considered (Foglietti 2020). This was confirmed during our fieldwork, as we discovered several traps and gunshells (Fig. 1.4) with most located on the forest edge, and encountered hunters or traces of their activity (e.g. campsites with recent fires, empty gunshells) in the deeper forest. Nevertheless, the presence of forest guards and patrols in the NP and law-enforcement are a deterrent.

Figure 1.4 Evidence of hunting practices in Gola RNP. Barbed wire traps and empty gun shells discovered during fieldwork in Gola south forest block. In most areas surveyed, it was likely to hear gunshots and observe campsites used by hunters. In a couple of occasions, we encountered hunter groups.

1.8.1.3 Community groups and livelihood

Gola RNP is in the territory of seven chiefdoms in the Kailahun, Kenema and Pujehun districts. By the 16th century, the region was mostly occupied by agriculturalists of Gola sociolinguistic group, who were more recently replaced by

Mende people who spread across southern Sierra Leone (Leach 1994). The original location of the settlements in Gola region is not clear in the literature, but probably due to the characteristics of the terrain (high elevation and dense forest) and a history of ethnic conflicts and civil war, people became established on the outskirts of the Park (Munro et al. 2013; Tubbs 2015). There is however, one report of depopulation of the region due to warfare around the turn of the century, and then being abandoned (Brncic et al. 2010). In Gola RNP, there was no forced displacement of local communities, unlike other regions of Africa that became protected areas.

Approximately 23,500 people live in the buffer zone and most identify as Mende (86%) and only 6.3% identifying as Gola sociolinguistic group. Over 50% of the population is under 18 years old, most people are Islamic believers (93%) and only 29% are literate (Bulte et al. 2013). Subsistence agriculture forms the basis of livelihood for 90% of FECs, with one quarter of the farmland being used for swamp rice production and the other quarter for profitable plantations of cocoa, oil palm and coffee (Bulte et al. 2013). Other products such as banana, pineapple, citrus and wood for charcoal are grown to be sold in the larger cities and the capital (Jusu and Sanchez 2013; Munro et al. 2013). Despite the short distance between FECs and Kenema (< 30 km), trade is limited by high transportation costs, difficult access as roads are gravel and become flooded during the rainy season, and in some cases not suitable for vehicles, and until recently, lack of mobile network for communication between stakeholders (Jusu and Sanchez 2014). Mende people use the forest for NTFPs collection, religious rituals and to visit former village grave sites (Bulte et al. 2013), but they also walk through it to reach the Liberian border and villages. Nevertheless, most NTFPs are collected in their home gardens, bush farms and the community forest rather than travelling long distances to the mature forest (Munro et al. 2013).

1.8.1.4 Conservation works

Gola RNP conservation management is coordinated by two conservation societies (RSPB and CSSL), one Sierra-Leonean Forestry division (Ministry of Agriculture, Forestry and Food Security) and the seven chiefdoms surrounding the forest reserve (Bulte et al. 2013). Logging, hunting, mining and farming are banned activities inside the park, while fishing and collection of NTFPs are still permitted. Law-enforcement ranger patrols have been deployed since 2005, covering around 7,000 km of the protected area per year (Barca et al. 2018a). Additionally, forest guards frequently conduct surveys and provide support to national and international research teams working in the forest. The presence of these entities reduces the chances of illegal activities taking place. Loggers, miners and hunters have been most affected by these conservation laws, however, farmers whose land is close to the protected forest have reported increased crop damage by monkeys, apes, buffalo and, more recently, elephants, likely due to easy access to fields and a ban on animal hunting (Bulte et al. 2013). Gola RNP management team is aware of the issues caused by such restrictions, and continuous efforts to improve community livelihoods through sustainable use of the forest are being investigated and implemented (e.g. a fair trade programme for cocoa production). Yet, regular research and monitoring suggest that conservation efforts and on-the-ground law enforcement are effective for biodiversity conservation, as is the case of the endangered western red colobus (*Piliocolobus badius badius*), whose populations appear to be increasing (Barca et al. 2018b).

1.8.2 Cantanhez National Park – Guinea-Bissau

1.8.2.1 The Republic of Guinea-Bissau

Guinea-Bissau is one of the smallest countries of West Africa (36,125 km^2) and comprises a continental mainland and an archipelago (the Bijagós). It is bordered by Senegal in the north, the Republic of Guinea in the east and south, and the Atlantic Ocean to the west (Fig. 1.2 b)). The most recent national survey in 2014 indicates a total population of 1.5 million with a sex ratio close to 1:1 (INE Guiné-Bissau 2014). Around 30 sociolinguistic groups of Muslim or African beliefs have been recorded in the country (Catarino et al. 2019). Fula (also known in other countries as Fulani, Fulbe, Peul or Pulaar) and Balanta groups are dominant, but all communities have a deep knowledge of the use of natural resources. Guinea-Bissau has a tropical climate, and the mainland territory is divided into coastal lowlands, interior plains and north-eastern highlands, with the highest point at 300 m (Hockings and Sousa 2013). The main vegetation types are mangroves, palm groves, woodland, savanna woodland and dry forest (Catarino et al. 2008), with a large proportion of the remaining West African primary subhumid forest in Cantanhez NP (Hockings and Sousa 2013; Catarino and Palminha 2018).

Political instability has exacerbated macroeconomic imbalances. Outside the capital of Bissau, the rural population has limited access to services and infrastructure (Catarino et al. 2019) and depend greatly on fishery and traditional agriculture, with rice cultivated in mangrove or forest soil, being the staple food (Sousa and Frazão-Moreira 2010; Sousa et al. 2014). Guinea-Bissau is a major exporter of unprocessed cashew nuts and many farmers depend on this cash crop for income (Barry et al. 2007). Other forms of income include NTFP trade in main markets, including the largest food market in the country (Bandim market in Bissau). A range of plants and animals originating from all over the country are sold as traditional foods and medicines (Minhós et al. 2013; Minhós et al. 2016; Catarino et al. 2019).

A network of protected areas has been developed since 1997, managed by the Instituto da Biodiversidade e das Áreas Protegidas (IBAP) (UNPD 2010). However, deforestation, soil erosion, overgrazing and overfishing are major threats to biodiversity conservation, as well as livelihoods and economic development (CIA 2010). Since 1970, Sahelisation (accumulation of salts originating from rain water, ocean water, or irrigation water) has increased temperatures and reduced precipitation, resulting in replacement of humid forested areas by dryer savanna-type vegetation (Catarino et al. 2016).

1.8.2.2 Geography, climate, biodiversity and threats

Cantanhez National Park was gazetted in 2008. It comprises the Cubucaré peninsula, in the administrative region of Tombali, south of Guinea-Bissau, and covers approximately 1,067 km^2 of territory. The NP boundaries (11º22’58’’N and

11º02’18’’N, and 14º46’12’’W and 15º15’58’’W) are delimited by freshwater rivers and the Atlantic Ocean, except an area in the southeast that borders with the Republic of Guinea (c. 20 km) and functions as a large mammal conservation corridor (Fig. 1.5). Prior to implementation, the region was affected by the war for independence, which took place between 1963 and 1974. The north of the park is easily accessed by a sealed road from the capital. Further south, a network of small, unsealed roads provides village access, but can be impacted during the rainy season, sometimes leaving the deepest areas of the park isolated for long periods of time. Travel is typically on foot, bicycles, or motorbikes that also serve as taxis.

Cantanhez is the southernmost of five protected areas in mainland Guinea-Bissau, containing a mosaic of subhumid forest, secondary forest, mangrove, savanna, human settlements, and agricultural fields (Gippoliti and Dell’Omo 1996; Catarino and Palminha 2018; Hockings et al. 2020). Further information on the main plant species found in each forest type can be found in Table A.1.2 (Appendix 1). This is the last place in the country where sub-humid forest can be found, a type of vegetation that covers parts of Guinea, Sierra Leone and Liberia, and that is characterised by large trees such as *Ceiba pentandra*, *Parinari excelsa*, *Sterculiaa africana*, *Dialium guineense* and *Ficus* spp., and several liana species like *Hunteria elliotii* and *Xylopia aethiopica* (Scott 1992; Rodrigues et al. unknown). One of the most common trees in the park that is exploited by human populations is the oil palm (*Elaeis guineensis*) and the cash crop cashew tree (*Anacardium occidentale*) (Catarino and Palminha 2018). Cantanhez has a marked rainy season between June and November, with the other months being mostly dry. Young foliage has a peak in production in July and August, but new leaves are available annually depending on plant species. Flowering is most common in March, with ripe fruiting happening between February and May (Bessa 2014).

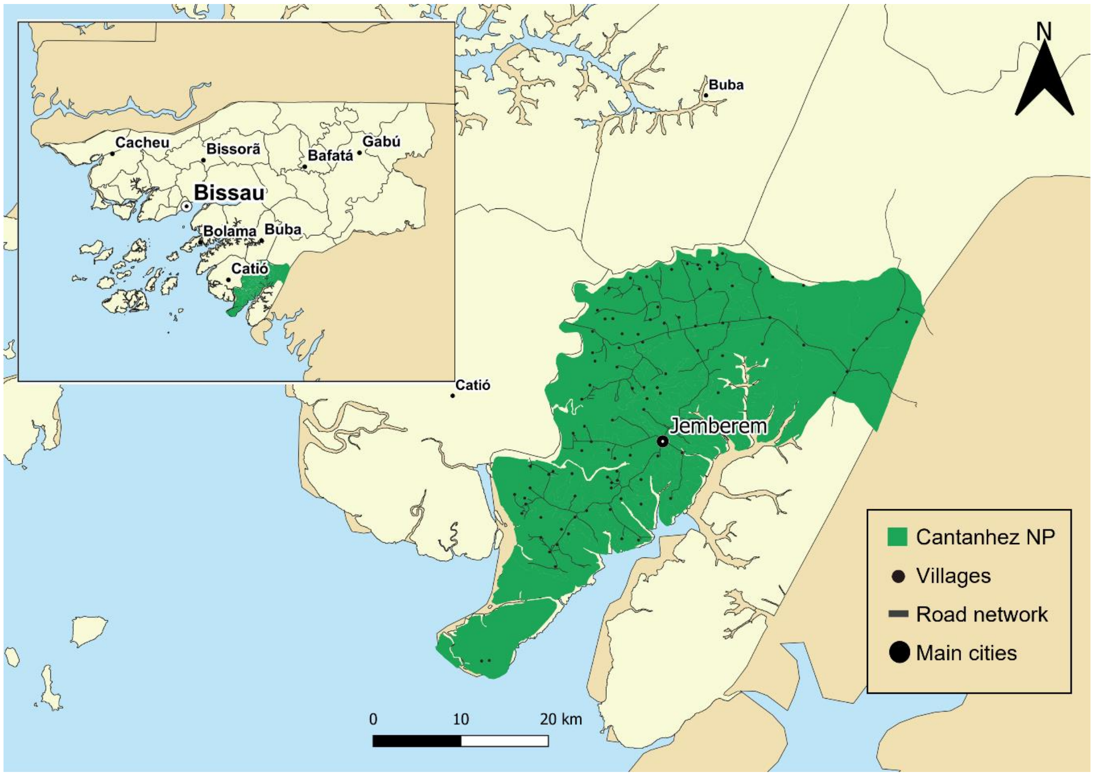


Figure 1.5 Cantanhez National Park map. The map shows the extent of the protected area (dark green) and the 110 villages located within it. For a detailed map on the types of vegetation present in the park, please refer to Fig. A.1.1 in Appendix 1. The main village is Jemberém, in the centre of the park. Upon field work conducted in this study, only the road at the top of the park was sealed, but since 2020, the road crossing the park vertically has been sealed until Jemberém. A series of smaller roads run through the protected area.

The remaining dense forest is reduced to 16 patches that have little anthropogenic intervention (Catarino and Palminha 2018). These can have different soil and vegetation, and can be under different conservation management due to traditional use by local communities (e.g. sacred forests). The park Management Plan has proposed division into three conservation areas (Table 1.1), where research and monitoring can take place.

Table 1.1 Three proposed areas of conservation in Cantanhez NP (Catarino and Palminha 2018).

Conservation area	Full preservation area (FPA)	Buffer zone (BF)	Sustainable development area (SDA)
Location	Patches of protected forest	Transition area between FPA and SDA	Adjacent to settlements
Protection level	High	Medium	Lowest
Human density	Low	Medium	High
Anthropogenic activities	Should be minimised	Some are allowed (e.g. NTFP harvest)	Traditional and sustainable practices

The heterogeneous ecosystems in Cantanhez NP harbour high biodiversity with 863 identified plants in the latest census (Catarino and Palminha 2018), several species of fish, birds, reptiles and mammals, of which seven are primates (western chimpanzee, Guinean baboon, western red colobus, King colobus, Campbell's guenon, green monkey and Senegalese bushbaby (Vieira et al. 2019; IBAP-PNC)). The main threats to biodiversity in Cantanhez NP include deforestation from slash and burn practices, bushmeat hunting, invasive species, and climate change (Catarino and Palminha 2018).

1.8.2.3 Community groups and livelihood

Several community groups have established in the Cubucaré peninsula. The first group documented to arrived in the 16th century were the Nalú, followed by Fula and Sosso in the 19th century, and from 1928 the Balanta established in the region (Catarino and Palminha 2018). The Nalú used the land for subsistence agriculture (including rice) and nowadays also grow fruit for sale. The Fula were mainly pastoralists initially (raised cattle and hunted), and later became fruit farmers and tradespeople, and the Balanta, who are traditional rice farmers in mangrove areas and are skilled at producing oil palm wine (Frazão-Moreira 2009; Temudo 2009; Catarino and Palminha 2018). Nowadays, there are approximately 10 sociolinguistic groups in the Cubucaré region with 62% identifying as Balanta, 10% Nalú, 8% Fula, and 20% as other groups (Catarino and Palminha 2018). The protected area accommodates approximately 24,000 people across 110 villages, with more than 50% inhabitants between 0-35 years old, and 42.3% literate (Catarino and Palminha 2018).

Locally, people consume fish, shellfish, rice and other crops, and bushmeat, despite it being illegal to hunt for endangered species within the park. Local communities also harvest botanical products for food, traditional medicine, construction materials, religious and traditional purposes. Tombali region was the main rice producer in the country but after the civil war, an expansion of orchards, mainly cashew nuts, took place as an alternative income for some communities (Temudo 1996). Even though small-scale orchards are rarely larger than 1 ha, more than 80% of the population farms and both forms of agriculture heavily contribute to forest degradation in Catanhez NP (IBAP 2007; Catarino and Palminha 2018).

In this National Park, the power of managing the forest and allocating land lies firstly in the hands of Nalú chiefs (Frazão-Moreira 2009). The Nalú are considered the founders of Cantanhez land, and so the territory is known as Nalú Land (or “chão Nalú”). The land is divided into (a) housing and cultivation ground belonging to each village; (b) “matos malgosse” located in dense forest areas, commonly used as worship sites but with restricted access and no resource extraction; and (c) forested buffer-areas surrounding the “matos malgosse”, where resource use is limited and logging is prohibited (Temudo 2009). More recently, there has been an influx of people into the region, not only from other parts of the country but also from the neighbouring Republic of Guinea, who are looking for work in the cashew and oil palm plantations (Catarino and Palminha 2018). This migration is currently seasonal, although some families have established permanently in the region. This increase in population density in the small peninsula of Tombali may have implications for Cantanhez forests and wildlife.

1.8.2.4 Conservation

Cantanhez NP is one of the last humid forests in West Africa and was identified as one of the seven priority areas for the conservation of the western chimpanzee (Kormos and Boesch 2003). After the implementation of Cantanhez as a National Park, central and local authorities along with the local population agreed to conserve the biodiversity of the area and develop income-generating ecotourism schemes benefiting the communities (Gippoliti and Dell'Omo 2003; Oliveira and Silva 2010). However, regulations are not enforced and formal protection for the forests and wildlife is limited (Hockings and Sousa 2013), hence hunting being a serious threat to local wildlife. In some study sites we found several traditional traps in the deeper forest, mainly aimed at ungulates (Fig. 1.6). Since 1990, several conservation and research programs have been developed, including work on local flora and fauna, zoonotic diseases, NTFPs and other sustainable livelihoods (Ecocantanhez), park socioeconomics, conservation (PRIMACTION), and the co-existence and co-use of plants between humans and chimpanzees (Catarino et al. 2020; The Darwin Initiative). For a summary of the main characteristics of each National Park, including differences in forest cover and composition, geography, and demography, refer to Table 1.2.

Figure 1.6 Hunting evidence in Cantanhez NP. Traditional trap on the ground (left) and empty gun shells (right) collected in the forest. Both evidence were observed in the north of the park.

Table 1.2 Summary of the main characteristics of both NPs. Geographic, demographic, ecological and conservation characteristics are summarised in the table for Gola Rainforest NP in Sierra Leone, and Cantanhez NP in Guinea-Bissau. Refer to section 1.7 Study sites for references.

National Park	**Gola Rainforest National Park (GRNP)**	**Cantanhez National Park (CNP)**
Official implementation	2011	2008
Location	Southeast of Sierra Leone	Southwest of Guinea Bissau
Forest type	Lowland moist evergreen forest	Sub-humid forest
Covering area	71,070 ha (~750km^2)	106,700 ha (~1,000km^2)
Rainfall	2500-3000mm/year	~2200mm/year
Rainy season	May - November	June - November
Altitude	< 426m	< 50m
Forest composition	Largely intact, except for previous logging areas: evergreen forest dominated by trees, moist semi-deciduous forest, freshwater inland swamp forest, forest regrowth and secondary forest, farmbush, herbaceous swamps and floodplains	Fragmented coastal forest: mosaic of dense and sub-humid forest, degraded secondary forest, mangrove, and savannah grassland, along with settlements, agricultural fields (cashew orchads, mangrove swamp and upland rice), and traditional oil palm areas.

Botanical survey	899 plant species	863 plant species
Soils	Granite hills, sandy clay loams, gravel terrain	Freshwater-marine, ferrous soil (iron)
Water bodies	Large freshwater rivers: Mano, Moro, Magbole, Mogbai & Mahoi; Koye river separates Gola south and central	Hydrographic bay (Rios Cumbijã and Cacine), brackish estuaries, freshwater river (Balana)
Conservation strategy	3 delimited areas under the REDD Project: (a) project area, (b) leakage belt, and (c) offsite zone	3 conservation areas: (a) full preservation area (16 forests or “matos”), (b) buffer zone and (c) durable development area
Populations	Adjacent to the park	Within the park
Settlements	122 forest edge communities (located in the 4 km wide buffer zone)	110 villages (200 if all settlements included)
Inhabitants	23,500 (> 50% below 18 years)	24,000 (> 50% below 35 years)
Ethnic groups	Mende, Gola; small no. Fula, Mandingo, Vai, Kissi, Limba, Gbandi, and Temne	Balanta, Nalú, Fula, Mandinga, Tanda, Sosso, Bijagó, Djacanca, Manjaco, Papél
Religions	Islamism (93.1%), Christianism (remaining)	Islamism, Traditional African Religions, Christianism
Literacy	29%	42%

Human migration	National and neighbouring Liberia migrants	National and neighbouring Guinea migrants
Roads	Few old logging roads inside the forest. One main gravel road crossing Gola south. Human-made paths.	A network of gravel roads inside the park, connecting the villages. Human-made paths.
Agriculture	90% of the population in the buffer zone	80% of the population
Chiefdoms or Territories	Malema, Gaura, Nomo, Tunkia, Koya, Barri, Makpele	Cabedú, Cadique, Iemberem and Guiledje
Activities permitted in the National Park	NTFP harvesting, fishing	Subsistence agriculture, NTFP harvesting, fishing.
Illegal activities	Site under pressure for exploitation of diamonds and iron ore. Bushmeat hunting and possible logging.	Deforestation, bushmeat hunting, pet trade
Conservation management	Strongly managed: patrols, forest guards and researchers	Limited in situ management
Human-wildlife interactions	Monkeys, apes, buffalos, elephants, among other mammals	Monkeys, apes, buffalos, among other mammals
Governmental Management Entity	Sierra-Leonean Forestry division, RSPB, CSSL and the seven chiefdoms where Gola RNP is located	Instituto da Biodiversidade e das Áreas Protegidas (IBAP)

1.9 Aims

The overarching goal of this thesis is to contribute to the evidence-based conservation of two endangered West African primates in two West African NPs, through the application of Next Generation Sequencing and ethnobotanical methods. This interdisciplinary project provides a crucial opportunity to combine methods from ecology, molecular biology and social anthropology to develop a more comprehensive understanding of (1) the use of wild plants by people living adjacent to a NP with a continuous and strongly managed evergreen rainforest in Sierra Leone, and communities settled within the NP with small sub-humid forest fragments surrounded by agricultural fields; (2) the influence of habitat disturbance on the dietary diversity of threatened primates, (3) the feeding flexibility of two primates with different dietary requirements and preferences, and (4) the use of wild botanical resources by primates and local people to evaluate levels of co-use.

1.10 Hypotheses and predictions

1) Botanical resources used by local human populations

    a. Study site social demography and conservation management strategies will have a direct impact on the frequency of use and salience of wild plants by rural communities.

        i. Population demography will contribute to variation in number of plants cited, with some groups, such as men, healers and people living in poorer chiefdoms, listing more wild plants than the other groups.

        ii. Increased distance to the protected forest and higher levels of amenities in the villages will result in lower levels of collecting of wild plants in the protected mature forest.

iii. Due to the position of villages in each NP, I predict that inhabitants around the Gola RNP in Sierra-Leone will demonstrate a lower level of wild plant use and knowledge than people living within Cantanhez NP, who are surrounded by habitats harbouring wild plants.

2) Variation in primate diet composition and species dietary flexibility

a. The diversity of plants in the diet of both primates will depend on the food availability in each landscape,

and

b. the capacity to adapt to a disturbed habitat will depend on the species' dietary preferences and ecological characteristics.

i. The DNA-based method will document diets with higher plant richness than detected in previous studies using traditional faecal analysis methods, for both primates. In Gola RNP, both primates will have higher dietary diversities and feed more on wild plants, while in Cantanhez, species richness of western red colobus and chimpanzees will be lower and species will include more cultivated foods in their diet.

ii. Seasonal climatic variation in tropical forests (e.g. changes in rainfall, temperature, photoperiod and phenology) implies changes in food availability. As a natural response, primates adapt their diets to feed on available food items that can provide enough nutrients to meet their dietary requirements. Hence, intraspecific variation across seasons, is expected to be observed in the diet of both primates in both NPs.

iii. Temporal analysis in western red colobus is likely to detect non-significant variation as the forest composition has not suffered major changes in the last decades, nor has it reduced in size. Vegetation shifts may have taken place but western red colobus are mobile species that can travel daily to feed on preferred available wild food items to maintain their nutritional balance.

iv. Dietary richness will be lower and niche breadth narrower for both primates in the disturbed fragmented forest of Cantanhez NP compared to the continuous forest in Gola RNP, but differences between parks will be more evident in the less flexible western red colobus. Dietary overlap between sympatric primates will be larger in Cantanhez NP compared to Gola RNP, due to poorer food availability.

v. Cultivated food items will be more frequently detected in both primates diet inhabiting Cantanhez NP compared to Gola RNP, but a higher prevalence will be identified in western chimpanzees due to their behavioural flexibility in anthropogenic landscapes.

3) Co-use overlap of wild plants by people and primates

a. Co-existence of sympatric humans and primates in an agroforestry environment contributes to a more extensive overlap of forest resources use.

i. Due to the social and ecological characteristics encountered in each NP, a more frequent relative co-use of wild plants is expected in the disturbed habitat of Cantanhez NP than in the undisturbed continuous forest of Gola RNP. As human communities in Gola RNP live outside the protected area and may have a lower use of the forest, the number of plants co-utilised by the forest dweller red colobus will be low. Chimpanzees, on the other hand, use other types of forest, and may be more present in the community forest (4 km buffer around the NP) where people cultivate crops. A higher level of overlap is

> expected between humans and chimpanzees, due to higher spatial overlap. In Cantanhez NP, as wild plants and forested area are reduced, both primates are compelled to use disturbed areas. As these areas are also used by people, a higher number of wild and cultivated plants utilised by sympatric humans and non-human primates is predicted to exist.

1.11 Thesis structure

This thesis was conceived and planned to enable an understanding of how humans and non-human primates use and share one of the most abundant and important natural resources, vascular plants, in a continuous and strongly managed habitat, and in a heavily anthropogenic degraded landscape. To reach the goals proposed in this project, non-invasive primate samples were collected in two West African NPs and the extracted DNA was sequenced for the universal plant marker Internal Transcriber Spacer (ITS2). Additionally, NPs human residents were interviewed using structured interviews and a freelisting technique to acquire information on wild plant use.

The latter aim is explored in Chapter 2. *Wild plants as a natural resource for human communities living within and outside West African protected areas*. By using qualitative and quantitative data, I was able to evaluate the importance of wild plants to local communities' livelihoods and determine which demographic factors contribute to the variation observed within each National Park. As the methods were standardised for both study sites, I was able to perform a cross-country comparison on the use of wild plants collected in the protected areas.

In Chapter 3. *DNA metabarcoding: a new tool that provides insight into the diet of the critically endangered Western chimpanzee (Pan troglodytes verus)*, a detailed dietary description is presented using a DNA-based method to identify plant taxa in non-invasive samples of a critically endangered ape, the western chimpanzee for the first time. I applied the high-throughput sequencing DNA metabarcoding approach using the universal ITS2 plant fragment in DNA because it is a more sensitive technique than traditional methods used in primate feeding ecology.

Chapter 4. *Ecological and temporal variation in the diet of the two western red colobus (Piliocolobus badius) subspecies* follows with an evaluation of the dietary variation of Bay red colobus populations (*P. b. badius*) in Gola RNP, Sierra Leone, and Temminck's red colobus (*P. b. temminckii*) in Cantanhez NP, Guinea-Bissau, in space and time, and using the same techniques as in Chapter 3. Differences between males and females are also evaluated for a small subset of the population living in the continuous rainforest.

In Chapter 5. *Wild plant overlap between sympatric humans and other primates in a continuous rainforest and an anthropogenic landscape*, I integrate the social and biological results to assess the extent of wild plant overlap in the diet of primates and local community livelihoods. By performing a relative comparison between the two NPs which show clear differences in settlement location and land use, I aim to present and discuss results that will contribute to evidence-based conservation of natural resources and threatened primates, ultimately benefiting rural communities.

Lastly, I summarise the main findings in the General Discussion, focusing on their conservation implications, and present future research and direction towards primate conservation in anthropogenic environments. An assessment of the limitations of the techniques and approaches used in this study are presented in the respective chapters. The product of each chapter will result in separate manuscripts.

CHAPTER 2. Wild plants as a natural resource for human communities living within and outside West African national parks

Palm oil fruits (*Elaeis guineenses*) and West African laburnum wood (*Cassia sieberiana*), forest products used by residents of Cantanhez National Park, Guinea-Bissau.

2.1 Abstract

In Africa, nearly half of the population occupy natural land, feed on wild foods, use plant resources for housing materials and energy, and depend on nature to obtain water. This nature-dependence is particularly prominent in rural areas due to the proximity to forested habitats and isolation from urban centres. Understanding where and when people use wild plants and for which purpose, is crucial for planning conservation and develop strategies that can allow for the sustainable use of wild plants without people losing their land tenure rights. In this investigation, two West Africa national parks served as case studies. Settlements in Gola Rainforest National Park, Sierra Leone, are located outside the protected forest and human activities are low, while in Cantanhez National Park, Guinea-Bissau, people live within the protected area and use the land to cultivate food species and other activities. To obtain information on wild plant harvesting, structured interviews with a freelisting technique were used (N = 52 in Gola; N = 56 in Cantanhez). A Salience score was calculated for each item listed, to determine which wild plants were more important. Levels of wild plant use between national parks did not differ significantly, suggesting that variation is a result of cultural differences and not other factors such as the location of villages. The presence of a community forest separating the villages and Gola forest, and which resembles the mature forest and provides most wild plants used, may explain the lower levels of plant resource use observed in the park itself. Additionally, the most salient plants in both parks were those with multiple purposes. Contrary to what has been proposed, residents in the wealthier chiefdom of Gola used more wild plants than respondents from poorer villages, but this difference could not be associated with the level of amenities per household. Some results, like this one, may have been influenced by people's perception of what is a legal activity within the protected forest. None of the variation could be assigned to specific demographic factors in Gola, and the travelling distance from the village to the mature forest did not influence people's use of wild plants either. Nevertheless, respondents interviewed in the rainy season listed nearly twice as many plants as than those

interviewed in the dry season. In Cantanhez, elders and people with Koranic education listed significantly more plants than the other groups, and variation in wild plant use among community groups was observed. These results are likely a reflection of knowledge that is acquired and transmitted across time and between people within the same group. This research presents detailed information on plant species which are important to people in each national park, and provides insight into reforestation knowledge and practices being carried out by some families or villagers. Both findings could be a good starting point for regional forest regeneration programmes involving local communities in the process.

2.2 Introduction

2.2.1 Forests as a natural resource

Global ecosystem health and the availability of natural products are intrinsically linked to societal wellbeing in the form of services or goods, such as water supply, climate regulation and raw materials (Costanza et al. 1997). Among the most widely used natural products are non-timber forest products (NTFPs), such as plants for food, medicine, and fibres, seeds, fruits and nuts, wood, palms and grasses, fish and game (CIFOR 2022; Wickens 1991). After World War II there was a shift in demand away from NTFPs to petroleum-based products, mainly in the international trade (Sills et al. 2011). At that time, the only NTFP of increasing interest was tropical timber (de Mello et al. 2020). It was not until the 1980s that forests were again recognised to provide a variety of products and ecosystem services to humans (UNCED 1987; Godoy and Bawa 1993; Costanza et al. 1997; Daily 1997).

Protected areas currently occupy 15% of the planet (UNEP-WCMC, & IUCN 2022) and besides prioritising ecosystem and biodiversity conservation, they should also protect local people and cultures through engagement into co-management and development initiatives, recognising the right of use of their territory and incorporating sustainable use of natural resources (Beltran and Phillips 2000). Many NTFPs grow in primary forests typically located in protected areas, of which approximately 98% overlap with territories belonging to traditional and indigenous communities (Paksi and Pyhälä 2018).

Around 1.2 billion people in the tropics highly depend on nature to meet their basic human needs, with 48% inhabitants of the African continent using nature and its products for at least three of the four needs: housing materials, water, energy or land occupation (Fedele et al. 2021). In the Lao People’s Democratic Republic, 80% of the population consume wild foods daily, while in India more than 50 million people depend on forests for subsistence (FAO 2013). In times of environmental and economic uncertainty, NTFPs provide security for rural households (Pierce and Emery 2005; Paumgarten 2007; Angelsen et al. 2014; Mugido and Shackleton 2017). Harvesting of NTFPs can be presented as an aid for the conservation of biodiversity and development of rural communities on a sustainable basis (Godoy and Bawa 1993; De Jong et al. 2000). However, the unsustainable use of NTFPs due to weak ecological management or the implementation of conservation policies non-inclusive of direct users has also been reported (de Mello et al. 2020), but it should be investigated using a site and species specific approach (Wollenberg et al. 1998).

2.2.2 Non-Timber Forest Products in West Africa

People use wild plants for a wide range of subsistence purposes and for generating cash income (Heubach et al. 2011; Mugido and Shackleton 2019). These resources are highly valuable in traditional medicine, in times of food scarcity, in the construction of houses and tools, and religious ceremonies (Belcher et al. 2005; FAO 2013; Bharucha and Pretty 2015; CIFOR 2022). The implementation of protected areas by historical colonial governments in Africa used a top-down strategy, displacing local communities to the outskirts of these parks and prohibiting natural resource harvesting in the conservation areas (Wilson et al. 2019). However, currently in West Africa, most NTFPs are freely accessible with few restrictions on collection and harvest (FAO 2001).

The oil palm, *Elaeis guineensis*, is one of the most heavily exploited trees in West Africa, with an estimated 10% of energy consumption in the region derived from its oil-based products (FAO 2001). Additionally, this plant has several other uses such as cooking oil, wood for construction, leaves for roofing and fences, and roots as medicines (Catarino et al. 2006; Carrere 2010). Other important plants found in the evergreen forests of Gola RNP are palm trees (*Eremospatha* spp.), and in habitats of Cantanhez NP are *Avicenna* spp. (in mangrove areas), and *Afzelia africana* and *Khaya senegalensis* (in gallery forests) (FAO 2001). Collection of wood as a fuel source has been linked to deforestation and desertification in West Africa, but it is less pronounced in the Guineo-Congolian region and the Guineo-Congolian/Sudanian transition zone, where Sierra Leone and Guinea-Bissau are located, respectively (Dounias et al. 2000).

Extensive ethnobotanical and floristic research has been conducted in West African countries, though little information is available for some countries (Dounias et al. 2000; Romeiras et al. 2012). The studies carried out in the forest area mainly comprise inventories, lists and descriptions of plants and their uses, while few studies globally have quantitatively examined the factors influencing anthropogenic wild plant use (Quinlan and Quinlan 2007). Most research in the two study sites have focused on NTFPs collected in all areas, including habitats outside the mature primary forest such as secondary forests, bush fallow or farm bush, and home gardens. Additionally, livelihood and NTFP valuation studies are frequently restricted to a small number of villages in one site only, or to a very specific domain (e.g. medicines to treat malaria), or involving at specialised respondents with a wide botanical knowledge (e.g. herbalists). This approach prevents international or inter-regional meta-analysis due to differences in the methodology used, or economic and governance contexts (Mugido and Shackleton 2019).

2.2.3 Study sites and demography

The case studies investigated in this study are two National Parks (NP) with conservation measures protecting endangered animal species, but that allow local communities to harvest NTFPs from the forest. Both NPs are Upper Guinean forests with high biodiversity levels, where humans and animals, such as non-human primates (hereafter primates), co-exist. The Gola Rainforest National Park (Gola RNP) in Sierra Leone is an almost continuous lowland moist evergreen rainforest with little anthropogenic presence and with villages located outside the protected area (Lindsell and Klop 2013). Cantanhez National Park comprises approximately 110 villages within its protected area, in a mosaic of sub-humid forest, secondary forest, mangrove, savanna and agricultural fields (Hockings et al. 2020). Communities from the regions of the two NPs are among the poorest households in the world ($1-1.25 per day) and thus depend greatly on NTFPs. In Sierra Leone, the main NTFPs are consumed for food and traditional medicine (Munro et al. 2013), while in Guinea-Bissau most people harvest edible plants and forage (FAO 2001).

Historical and demographic characteristics in both sites are similar. Both have undergone recent civil wars and experience immigration from neighbouring countries. Forest use by residents is overseen by the national park authorities. More than half of the inhabitants are younger than 18 years old (Bulte et al. 2013; INE 2017), and live in patriarchal and hierarchical societies, yielding differences in the characteristics and capabilities of each gender and social age (Leach 1994). Young and elders are the dependent groups as their contribution to daily tasks and income generation is smaller. Among adults, tasks are differentiated between women and men (Frazão-Moreira 2009; Bulte et al. 2013).

2.2.4 Aims and hypothesis

In this thesis, we included a broad range of respondents across a wide geographic area, using a freelisting methodology that yields insights into community knowledge and importance of a cultural domain, wild plants in this case (Bernard 2006; Newing

2011; Albuquerque et al. 2014). Hence, this is a suitable technique to perform a quantitative comparison of two or more communities. Using a freelisting approach in the interviews, this study aims to identify and elucidate the social and demographic drivers of wild plant use by local communities within each NP and between the two NPs.

Accordingly, we present the following hypotheses:

1) Demographic factors:
    a. *Gender* – Living in patriarchal and hierarchical societies yields differences in the characteristics and capabilities of each sex (Leach 1994). In Gola RNP, men tend to travel farther and sometimes alone to the protected forest, and they perform tasks that involve spending more time in the deeper and mature forest than women (Bulte et al. 2013), so I predict that men will use more wild plants than women. This is less likely to be the case in Cantanhez NP, as women also harvest for fire wood, and fish and seafood which involves travelling through the forest to reach the coast where these products are found (Frazão-Moreira 2009).
    b. *Age* – Older people generally have more experience of using wild plants to perform different activities, and they may have also gained more knowledge about the uses of wild plants from observations and/or oral testimonies (Quinlan and Quinlan 2007; Frazão-Moreira 2009; Constant and Tshisikhawe 2018; Heineberg and Hanazaki 2019). In this study, I expect elders to list more wild plants than adults and young. Even though they may no longer collect the plants themselves, they will still use them when collected by others.
    c. *Education* – In some rural communities, attending school requires going to towns and becoming more acculturated. As a result, more educated people tend to choose biomedicine over traditional

medicine (Zent 2001; Quinlan and Quinlan 2007) and consequently have reduced knowledge about "bush medicine". Additionally, NTFPs may provide a valuable ecosystem service for the poorest and oldest in communities (Sills et al. 2011), increasing their knowledge and use of such products. In rural Sierra Leone, most official schools are located in urban centres distant from the villages, and transportation is limited and costly, as well as primary education itself (Leach 1994; Bulte et al. 2013). Considering these social characteristics, I predict that people who attended official school will use less wild plants than those who did not or had Koranic education (taught by an Imam in the village, no travelling required). Contrary to Gola RNP, I do not expect to discover any variation between people who did and did not attend school in Cantanhez NP, because both official and Koranic educational opportunities are more easily accessible. Furthermore, considerable enhancement of an individual's social and cultural knowledge (including natural resources) is obtained through the initiation rituals still commonly practised in the Cantanhez region (Frazão-Moreira 2009).

   d. *Community group* – There are several community groups in Cantanhez NP with individual histories, cultures, practices and preferences (Temudo 2009; Salvaterra and Casanova 2020). As a result, we predict variations in the plant species used but not in the number of plants free listed.

2) Geographic factors:

   a. *Forest block* – Villages in Makpele chiefdom (Gola south forest block) have more amenities (e.g. electricity and toilet facilities) than villages in other chiefdoms. This is probably a result of living in a high diamond mining activity region close to the Liberian border and crossed by the Monrovia-Freetown road, which enhances cross-border trade (Bulte et al 2013). Hence, we predict that wealth differences between Gola chiefdoms will influence wild plant use. Residents of Makpele chiefdom will likely list less plant items than people from Gaura and Malema chiefdoms (Gola central).

b. *Distance to the protected forest* – Most NTFPs in Gola RNP have been reported to be collected mostly in the community forest surrounding the villages (Munro et al. 2013). We expect residents who live further away from the mature forest to list fewer wild plants in their interviews as they would only travel far when a specific product that cannot be found in the community forest is required.

c. *Place of residence* – Considering village location in each NP, I expect a general higher use of plants in Cantanhez NP as respondents live within the protected forest, while in Gola RNP, all interviewees live within four kilometers of the protected forest border, requiring longer periods of travelling to reach wild plant resources.

Ultimately, this study will provide baseline information on the use and type of wild plants collected by residents of two National Parks, that will be integrated in a cross-disciplinary analysis to understand the extent of habitat co-use and co-existence with primates.

2.3 Methods

This chapter is based on the outcomes of interviews with a wide range of people living within or on the edge of each National Park. I used structured interviews that included a section where people could freely list the wild plants they use and that are collected in the protected areas. Respondents comprised both men and women of different ages, performing diverse activities, with varying levels of education, and living in villages at a range of distances from the protected forest. The broad spectrum of demographic and geographical characteristics should enable detection of variation in wild plant use across the populations surveyed and provide insights into the importance of wild plants for regional livelihoods. The freelisting technique was used in this thesis as a way to identify the most salient wild plants that are used by local residents in each National Park, and allow comparison between parks that implement different conservation management strategies. Ultimately this will reveal the extent of overlap between plant species used by humans and those present in the diet of the western red colobus and western chimpanzee.

Additionally, our findings in Sierra Leone and Guinea-Bissau will provide baseline data on wild plant use by both humans and primates, and the differences between a better-preserved forest with low levels of human presence (Gola RNP in Sierra Leone) and a more disturbed and highly populated protected area (Cantanhez NP in Guinea-Bissau). Full descriptions of study sites and human community groups can be found in Chapter 1, section 1.7. Further information on data collection and subsequent analysis used in this chapter will be described in turn.

2.3.1 Data collection

Information regarding the human populations of each national park was collected during the interviews, in reports made available by park authorities (Gola RNP and Cantanhez NP), and literature from previous regional studies. For the independent variable "distance from the village to the protected area" in Gola RNP, we standardised distance by measuring the shortest straight line from the village to the forest boundary in QGIS (*QGIS.org* 2022). This may not be the exact path that people take to reach the protected forest, but we did not accompany people during this activity and so did not gather this precise information.

2.3.1.1 Selection of villages and respondents

I used the non-probabilistic cluster technique to conduct interviews in villages, selected for proximity to areas where primate biological samples had been collected (see Fig. 3.3 and Fig. 3.4 in Chapter 3, section 3.3 for more details on biological sampling and village location). Within each village, I then used a stratified random sampling approach with the aim of interviewing equal numbers of men and women in three age cohorts (social age: elder, adult, young). Using two independent variables (sex, age) in the sampling design allows me to maximise the variance between groups and minimise within each group, thereby reducing sampling error (Bernard 2006; Newing 2011; Albuquerque et al. 2014). In the months of May, June and November of 2018, a total of 52 structured interviews were conducted in 15 villages located in the leakage belt of Gola Rainforest National Park (RNP) in southeast Sierra Leone (Table 2.1). Interview questions were initially piloted with six interviewees prior to data collection, and some revisions on sentence construction for better understanding were made to the script (e.g. National park was replaced by protected forest).

Sierra Leonean research fieldwork assistants hired by our team to develop work during the period of research, were employees of Gola RNP. They were all men between 40 and 50 years old, originating from urban areas, with long experience in Conservation programs on biological and social data collection. They always wore a form of identity during working hours and in some cases their association with the Park entity might have had an influence on interviewees answers, especially when questioned about permission to use the forest plant resources. Despite the research assistants being known in many local villages, we always hired a community member who is involved with conservation projects undergoing in Gola RNP, due to their connection with the local town chiefs and great knowledge of the forest. Both research and community assistants acted as interpreters when interviewees did not speak English (majority of the cases). Once again, the fact that interviewees were familiar with the assistants, that informants knew they were associated with Gola RNP, and that they translated the questions, may have created a bias in the answers provided by the informants. However, the people who enforce the laws inside the protected area are the Park rangers, with whom we never worked.

In the following year, during March and April 2019, 56 residents from 12 villages within Cantanhez National Park (NP) in Guinea-Bissau, were selected for interview (Table 2.2). In this NP, the connection with the Park director and its guards was purely bureaucratic. To join me in the fieldwork, I hired two community members who have been working with international researchers for more than 10 years, collecting biological and social data for Anthropological, Ethnobotanical and primate conservation research. They were two men between 35 and 40 years old, well known in the region, with important connection with local town chiefs and residents. At the time, a Guinean-Bissau undergraduate female student in her early twenties, contributed with her time as a volunteer to this research, and was trained to conduct interviews by herself, as she was fluent in the country's creole. All three members translated the questions when Portuguese was not spoken by the informant.

Table 2.1 Number of interviews conducted in Gola RNP (N = 52) per forest edge community (FEC). Information on the 15 villages location, population size, number of houses and number of respondents is presented. FECs are separated by forest block, as Makpele chiefdom is wealthier than the other two chiefdoms in Gola Central.

Forest block	Village chiefdom	FEC surveyed (total FEC per chiefdom)	Pop. 2010[a]	No. houses[b]	No. respondents
Gola central					36
	Gaura	5 (9)			(12)
		Gbahama	47	12	4
		Genneh	363	44	2
		Gombu	500	57	1
		Lalehun	600	55	2
		Nyawama	305	46	3
	Malema	5 (13)			(24)
		Bandajuma	109	17	6
		Jagolahun	25	10	6
		Patama	na	10	1
		Taiama	250	32	5
		Takpoima	150	18	6
Gola south					24
	Makpele	5 (32)			(16)
		Dombu	550	65	4
		Jeneva	25	5	2
		Ngekpa	na	na	2
		Nyeyama	na	na	4
		Pewaa	780	95	4
Grand total:		15	23,500	-	52

[a] Census data from Bulte et al. 2013. [b] number of houses acquired in GRNP database (shapefiles provided by Gola RNP GIS department).

Interview scripts used in both countries were written according to the local culture and characteristics of each National Park (Script A.2.1 and Script A.2.2 in Appendix 2). In Sierra Leone, we were able to choose the same number of villages (five) across the three chiefdoms where biological sampling had taken place (Gaura, Makpele and Malema, Table 2.1). However, in Guinea-Bissau, fieldwork constraints prohibited this approach (Table 2.2).

Table 2.2 Number of interviews conducted in Cantanhez NP (N = 56) per village. Information on the 12 villages location, population size, number of respondents and prevalent community group interviewed is presented. Villages are grouped by chiefdom, with Guiledje villages being located in the north of the park, and Cabante the most southern village interviewed.

Village chiefdom	Villages surveyed	Pop. 2009[a]	No. of respondents	Community group
Cabedú			(5)	
	Cabante	110	5	nl
Cadique			(44)	
	Cadique	322	4	nl
	Caiquene	141	4	nl
	Cambeque	74	4	td
	Canamine	228	8	ba, fu, td
	Cassintcha	107	3	ss, bj
	Catombói	88	6	nl
	Jemberém	992	3	td
	Lautchande	76	3	ba
	Madina	229	9	ba, bf, md, ss, fu
Guiledje			(7)	
	Áfia	262	4	fu
	Quebo-Sutuba	302	3	ba, fu
Grand total:	12	24,293	56	

[a] Census from INE (2017). Community group: community groups interviewed in the village – ba (balanta), bf (biafada), bj (bijagó), fu (fula), md (mandinga), nl (nalú), ss (sosso), td (tanda).

In both countries, the procedure to approach people and conduct interviews in a chosen village was identical. The fieldwork assistants or interpreters would contact the town chief prior to our visit. If the town chief agreed to receive us, on the arranged day and time, the town chief would invite the research team to his/her house or outdoor space for an informal conversation, whereupon the team would present the purpose and objectives of the project. On approval to carry out the research, the town chief would be interviewed, and the next respondents would be chosen according to their availability at the time. On some occasions, respondents were identified by the town chief, and when villagers were away working on farms, we rearranged the interviews for another time or day. The same introductory explanation was given to each respondent, and if their informed consent would be given (orally), the interview would be conducted. The respondent was given the information prior to initiating the interview, that they could withdraw at any point with no consequences, and the information provided till that point would be discarded and not used in the research. Upon concluding the interview, respondents were given time to ask any further questions about the project (refer to section 2.3.2 for Ethical considerations).

The desired number of interviewed men and women in the three age cohorts was not always met due to the socio-demographics of each village, and availability of villagers during farming season (Fig. 2.1, more details in Tables A.2.1 and A.2.2 in Appendix 2).

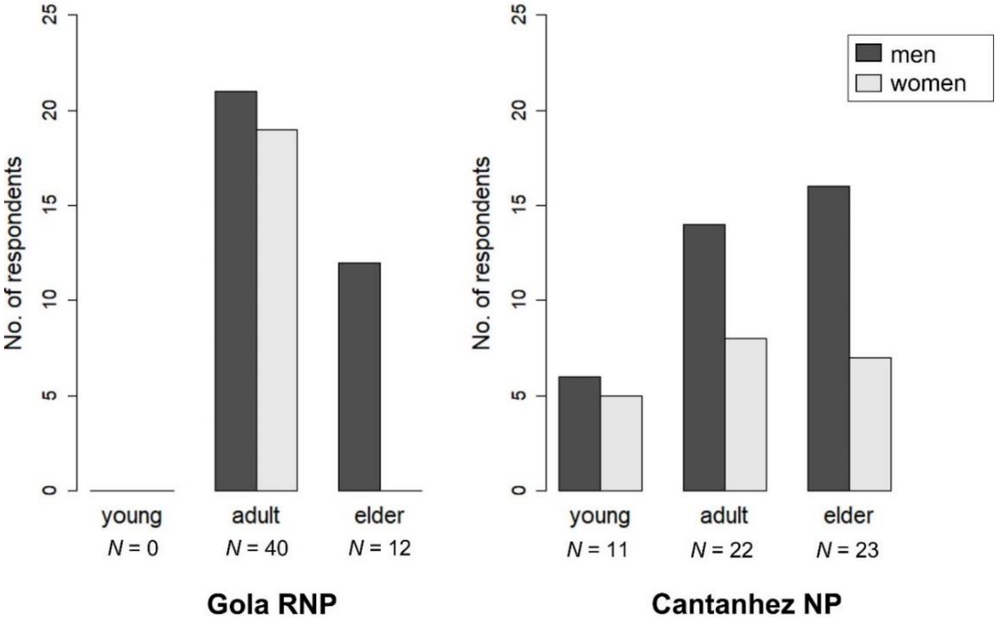


Figure 2.1 Number of women and men interviewed in each age cohort in Gola RNP and Cantanhez NP. Although the same number of interviews per gender and per age cohort was aimed, people’s availability did not permit similar number across categories.

2.3.1.2 Structured interviews

The oral interview was preceded by socio-demographic questions about the respondent. This was designed to obtain information on wild plants used by the respondent that are collected solely on protected grounds: i) reasons for collecting wild plants, ii) permission to collect forest botanical products, iii) who collects the plants, iv) time/season of collection, v) its broad uses, vi) where the plants are found, and vii) whether they have previously planted these items in their home gardens, bush or fallow, and orchards. The word ‘garden’ will be used in this work to refer to all these places where people actively cultivate or manage plants, and where they could possibly grow wild plants from seeds collected in the mature dense forest. The structured interviews (Scripts A.2.1 and A.2.2 in Appendix 2) included closed- and open-ended questions, and a section where respondents could list freely the wild plants collected in the national protected area (the Freelisting method – see section 1.3.1.2).

Few interviews were conducted in the national language (English in Sierra Leone and Portuguese in Guinea-Bissau). Most were performed in the *lingua franca* (Mende in Gola RNP, Creole in Cantanhez NP) or any other language spoken in the park, contingent on fluency of both the interpreter and the respondent. The interpreters who facilitated the interviews in non-official languages had access to the script beforehand to familiarise themselves with the questions and clarify any doubts. The translators/field assistants were experienced working with researchers and respondents in past research projects. In Guinea-Bissau, although the field assistants collaborate with the Cantanhez NP rangers’ team, they do not have a working contract/link with the Park. In contrast, for Gola RNP, all field assistants and interpreters were park rangers or temporary field guides hired for specific projects developed under the park management.

In both sites, although most people are accustomed to the presence of researchers in their villages, our visit was a welcome event and often a group of people would gather to listen to the first interview. We attempted to avoid bystander contamination, whereby onlookers can influence interviewee responses, by restricting each interview to one member or household. Accordingly, we did not include any contribution that was made by any person other than the respondent. We also avoided interviewing people who had assisted with previous interviews. For each interview, we coded the script to guarantee anonymity and performed descriptive statistical analysis for each population (Gola RNP and Cantanhez NP).

2.3.1.3 Freelisting of wild plants

Freelisting is a rapid and easy interview technique commonly used in social sciences to assess a specific area of knowledge (cultural domain) in a population (Albuquerque et al. 2014; Chaves et al. 2019). Interviewees list items relative to the domain in question, and the data extracted can inform on four main points: community familiarity with the items, participant knowledge of the research topic, the most important items locally (item salience), and individuals with greater knowledge on a specific cultural domain (Quinlan 2005; Newing 2011). In principle, culturally important items will be listed more frequently across interviews and ranked higher by respondents (Albuquerque et al. 2014), enabling comparison between two or more communities using a specific software (ANTHROPAC® (Borgatti 1996), and AnthroTools R package (Jamieson-Lane and Purzycki 2016). The informatic tools combine the frequency of citation of each element mentioned in the interviews and its average position on the list to determine the relative importance of items (salience score or Smith’s S) (Smith 1993).

In this study, we implemented this technique to rapidly assess anthropogenic wild plant use for multiple purposes (e.g. nutrition; construction of houses, boats, and tools; traditional medicine; religious). During the interviews, respondents listed wild plants they use, either those collected by themselves or someone else, in the forest area protected under the National Park. We used lists of vernacular names for local plants produced in previous regional ethnobotanical studies, as prompts for identification of items *in situ*. In some cases, we asked the interviewee to show the item if they had it in their homes or gardens, or to identify the plant species using a plant guide (Arbonnier 2002). Confirmation of plants listed and scientific names were later obtained using botany books from West Africa (Savill and Fox 1967; Catarino et al. 2006; Hawthorne and Jongkind 2006; Catarino et al. 2020) or expert knowledge (Mr. Mohamed Swaray for Gola RNP plants).

When a respondent finished listing items, we would read back the list. This is an interviewing technique that reminds the respondent what was listed and allows for additional items, potentially increasing the total number of items listed (Brewer 2002). To maximise information on wild plants collected in protected areas, the aim was to interview people until theoretical saturation of the answers (Bernard 2006; Newing 2011), when new respondents did not list any new plant items. However, this was only partly possible in Gola RNP and not achieved in Cantanhez NP due either to respondent unavailability when working in distant farms or the small number of households in a village. Occasionally, we continued conducting interviews after reaching zero new items listed because the villagers insisted on being interviewed. To perform further analysis (salience of items listed, the most important or most used items), each plant item was given a position number (rank) according to the order it was cited by the respondent. Number 1 is given to the first item mentioned, number 2 to the second, and so on. We did not limit the items that could be listed in order to obtain as much information on the domain as possible.

2.3.2 Ethical considerations

Study and interview design were approved by the ethics committee of the Centre for Research in Anthropology (CRIA) in Portugal, and BIOSI Ethics at Cardiff University, U.K., and comply with The European Code of Conduct for Research Integrity. Prior to performing interviews, I obtained formal permission from the Research and Monitoring Department of the Gola RNP in Kenema, Sierra Leone, to conduct the research in Gola region, and from Instituto da Biodiversidade e Áreas

Protegidas (IBAP) in Bissau, Guinea-Bissau, to interview residents in Cantanhez NP. After informal conversations with town chiefs, where we explained the nature of the research, the objectives of the study and how the data would later be used (Documents A.2.1 and A.2.2 in Appendix 2), we clarified what was expected from the respondents, and guaranteed anonymity and confidentiality of the answers. We answered any further questions from respondents. We only continued interviews with oral agreement from respondents. We did not require written consent, due to cultural considerations and/or low literacy. Some questions involved sensitive issues related to illegal activities performed in the protected National Park. Therefore, to avoid compromising interviewees or deterring participation for fear of being reported to the authorities, interviews were not audio recorded. Throughout this thesis I use coded data to safeguard respondent identity, but in some cases, respondent age group, school level, activity or village may be indicated.

2.3.3 Social demographics

In 2018, we conducted 52 interviews across 15 villages on the edge of Gola RNP, of which 38 were during the dry season (May and June) and 14 during the rainy season (November). In 2019, we conducted 56 interviews in 12 villages in Cantanhez NP, all during the dry season (March and April). Tables A.2.1 and A.2.2 in Appendix 2 provide detailed demographic information per village. Each interview was coded and transcribed to a Microsoft Excel spreadsheet. Interview scripts can be consulted in Scripts A.2.1 and A.2.2 in Appendix 2. By using statistical analysis on the data collected on the sociodemographic and closed-ended questions, we were able to characterise the populations surveyed in this study.

Case study 1. Gola Rainforest National Park, Sierra Leone

Gola region is predominantly inhabited by Islamic Mende people who depend on farming activities to sustain the family (Bulte et al. 2013). Our sample reflects this, with nearly two thirds of the respondents being male (63.5%) and over two thirds being adult (67.3%). Exact age or social age (elder, adult, young) was self-reported by the respondent. The latter reflects either their position in the society or their life stage (e.g. town chief, if married, with children). For analysis, I used the variable social age, and allocated people who reported their age in years to the following cohorts: dependent categories - young < 15 years and elder 65^+ years, and the working age group - adult between 15-64 years (as in *SLIHS* 2019). A small percentage of respondents attended either Koranic (19.2%) or official schools (7.7%), illustrating the low literacy characteristic of the area. Crop plantations are a common source of income, and some respondents owned cocoa, coffee, pineapple, banana or kola nut plantations, or rice fields. None had large farms. Only two of 52 interviewees indicated their main occupation as "healers"; yet of the 35 who use wild plants, more than 70% use the items in traditional medicine. Cited plants were collected in the protected forest, where people travel between 300 m and 4 km each way to collect the natural products. Sierra Leone has one of the poorest primary healthcare and family medicine services among the least developed countries (Robinson 2019), which was exacerbated by the Ebola outbreak in 2014 as 7% of total deaths were healthcare workers. For a population of 7.4 million people (62% living in rural areas), there are only 1000 doctors, nurses and midwives, and three referral hospitals in the country, and the gap between urban and rural care is considerable (70% of the health workforce is present in urban centres – *HRHS* 2017). Despite there being a community health programme with centres in rural areas that provide basic care, people living in the districts around Gola RNP must travel more than half a day to reach the nearest health clinic (Community survey 2010 in Bulte et al. 2013). Additionally, most of the population must pay their own expenses and therefore, it is typical for Sierra Leoneans to use wild plants for initial treatment (Robinson 2019). Likewise, urban centres where people have easy access to more modern products, are distant from most villages, except in Makpele chiefdom (mining region and close to the Liberian boarder) (Bulte et al. 2013).

Case study 2. Cantanhez National Park, Guinea-Bissau

The respondents in our Cantanhez NP sample identified themselves as Nalú (33.9%), Balanta (21.4%), Fula (14.3%) and Tanda (14.3%). The remaining people were from the Sosso, Biafada, Mandinga and Bijagó communities. Most interviewees (96.4%) were farmers or people whose main occupation involves spending time in the forest area (e.g. carpentry, salt mining, oyster harvesting). Their farms are used to grow vegetables for their household and extended family. None of the respondents held a commercial farming business but some had other plots of land to grow cash crops such as cashew, and other explored palm oil areas, a supplementary income to support the family. More than one third of the respondents were women (36.6%), and 41.1% of the interviewees were elder and 19.4% young. Only two of the 12 villages we interviewed had schools, so most children had to travel to the nearest school on foot. Yet, 69.6% of the respondents had some form of education (official school, 58.9%; Arabic school, 10.7%) with 37.5% reaching secondary school. In our sample, we interviewed seven people who are healers and therefore use local wild plants for treatments. Islamism is the main religion in the park, reflected by our interviews; 78.6% of the respondents were Muslim, followed by 12.5% Balanta (traditional African religion), 5.4% Evangelic (strong presence of charity groups in the area) and 3.6% Christian.

2.3.4 Data analyses: qualitative and quantitative

2.3.4.1 Patterns in the data and wild plant choice

The combination of qualitative and quantitative methods aimed at searching for patterns that could test the hypothesis proposed. I examined the responses of a total of 108 interviews conducted across both national parks and summarised important ideas through direct and indirect quotes. Percentage of respondents, trends, and associations between factors (dependent and independent variables) were analysed with statistical methods available in R3.6.1 (*R Core Team* 2022). I used a two-tailed, two sample t-test (test statistic: t), a Mann-Whitney U test (test statistic: W) or a Kruskal-Wallis test (test statistic: X^2) to test for the significance of differences between users and non-users of wild plants, and to determine whether the number of plants listed differed between the groups of each category. For this thesis I decided not to include multivariate tests because sample size for some villages is low, and this would reduce the number of comparisons that could be performed. However, this type of tests will be considered for future publication of the results. I performed correlation analysis to test for associations between the dependent variable (number of wild plants cited) and distance to the protected area, using Pearson's or Spearman's rank methods.

2.3.4.2 Salience Index

To estimate the salience (most important or cited) of each plant item using qualitative data, I performed a Salience analysis (Smith 1993) using the AnthroTools package (Jamieson-Lane and Purzycki 2016) in R3.6.1 (*R Core Team* 2022). For the data obtained in Gola RNP, I used the answers of the interviewees that use plants (N = 35), excluding the non-users. For Cantanhez NP, I used all 56 interviews as all respondents used wild plants. Although we could not identify some items to species level, these were kept throughout the Salience analysis because it has a clear importance to the respondent. Nevertheless, the mean frequency of mention for these plants is 1.4 (SD = 0.7). Salience analysis elucidates which items are most important for each individual, and how much variation in wild plant use exists in each region. The salience of each item cited in each freelist is calculated by inverting the order (ranking position of the item for each respondent) in which the item was cited (k) and dividing this value by the total number of items cited during the interview (n) (Purzycki and Jamieson-Lane 2017):

$$\text{Item salience} = \frac{(\mathrm{n} + 1 - \mathrm{k})}{\mathrm{n}}$$

We then calculated the composite Salience (Smith's S) for each item listed across all interviews, by adding all salience scores for that specific item and dividing by the total number of respondents interviewed, including those that have not listed that specific wild plant. Items with higher Smith's salience are generally listed more often and earlier in respondent freelists (Quinlan 2005). We could further identify the most salient plants for group (gender, age cohort, occupation, etc.) by comparing the composite salience values of each plant item.

2.4 Results

2.4.1 Gola Rainforest National Park

Wild plant use

A majority of interviewees (67.3%) claimed to use plants that were collected in the protected area of Gola rainforest. Respondents living in Gola south were more likely to use wild plants collected in the protected area than respondents residing in Gola central (100% users in Gola south, 52.8% users in Gola central; Fisher's exact test, N = 52, $p < 0.001$). Not all interviewees agreed that one of their rights as residents of the premises of Gola RNP is to collect non-timber forest products (NTFPs) within the protected area (Table 2.3). Gola RNP is a dense forest that is protected by armed guards, and the presence of researchers and assistants is common. An adult from Gaura chiefdom (in Gola central) reported:

> *I used to go to Gola* [protected forest] *but not anymore because I am old and the forest is too far. I know I am allowed to go to Gola but I am afraid because the armed forces go along with the rangers and they don't ask anything, they just shoot.*
>
> [Respondent SL41 - 03.11.2018]

Thirteen respondents (30.2%) reported being forbidden from collecting wild plants, and some claimed that *Gola is protected and there are laws that forbid it* [collecting plants], while others suggested that *they will be arrested if they collect plants from Gola protected area*. In fact, community perception of their rights of forest use significantly influences the choice of using wild plants among the interviewees (Fisher's exact test, N = 43, $p < 0.01$) (Table 2.3). All respondents that do not use wild plants and believe they are forbidden to collect this product from the protected area, reside in Gola central.

Table 2.3 Utilisation and perception of right of use of wild plants. People had differing opinions on the right to harvest wild botanic products from the protected area (PA) in Gola RNP, but that did not deter some respondents from using it.

	use wild plants			do not use wild plants		
Number of respondents who...	Gola central	Gola south	Total (N = 43)	Gola central	Gola south	Total (N = 43)
believe are allowed to collect in PA	17	6	23 (53.5%)	7	0	7 (16.3%)
believe are forbidden to collect in PA	2	2	4 (9.3%)	9	0	9 (20.9%)
Total (N = 43)	19 (44.2%)	8 (18.6%)	-	16 (37.2%)	0 (0%)	-
did not answer	0	8	8	1	0	1
Grand total (N = 52)	19	16	35 (67.3%)	17	0	17 (32.7%)

Plants are collected by the respondent (57.1%) or another person (42.9%), but no significant differences in the mean number of plants were detected between the two groups (respondent: 4.8, other: 5.0, t = -0.254, d.f. = 33, $p > 0.05$). In general, men carry out this task (62.9%) as they are the ones who use the forest more frequently, or because women are *"afraid of going to the forest"* or the forest is *"too far"*. Of the 35 respondents who use wild plants for domestic purposes, 11.5% also commercialised a part of the plant products. As our interviews were not targeted at key respondents in the domain of wild plants, we only interviewed two people who practise traditional medicine, both of whom were adult males. The first was a healer with several other occupations, and listed a total of four plants. This is similar to the average used among farmers (4.7, N = 30) and miners (4.5, N = 2). Contrastingly, the second respondent was a “certified” healer, with farming as a secondary occupation, and listed a total of 12 plants, eight of which were unique (only listed once across all interviews).

> *My Arabic teacher taught me to be a herbalist. I had a document* [proving a certified healer], *but during the war they burnt all documents*. [Respondent SL46 - 06.11.2018]

In total, 49 plant items were recorded and 44 were identified to species level (comprising 39 genera and 24 families). Fabaceae, Rubiaceae and Arecaceae were the plant families with most items listed (6, 5 and 4, respectively; Fig. 2.2).

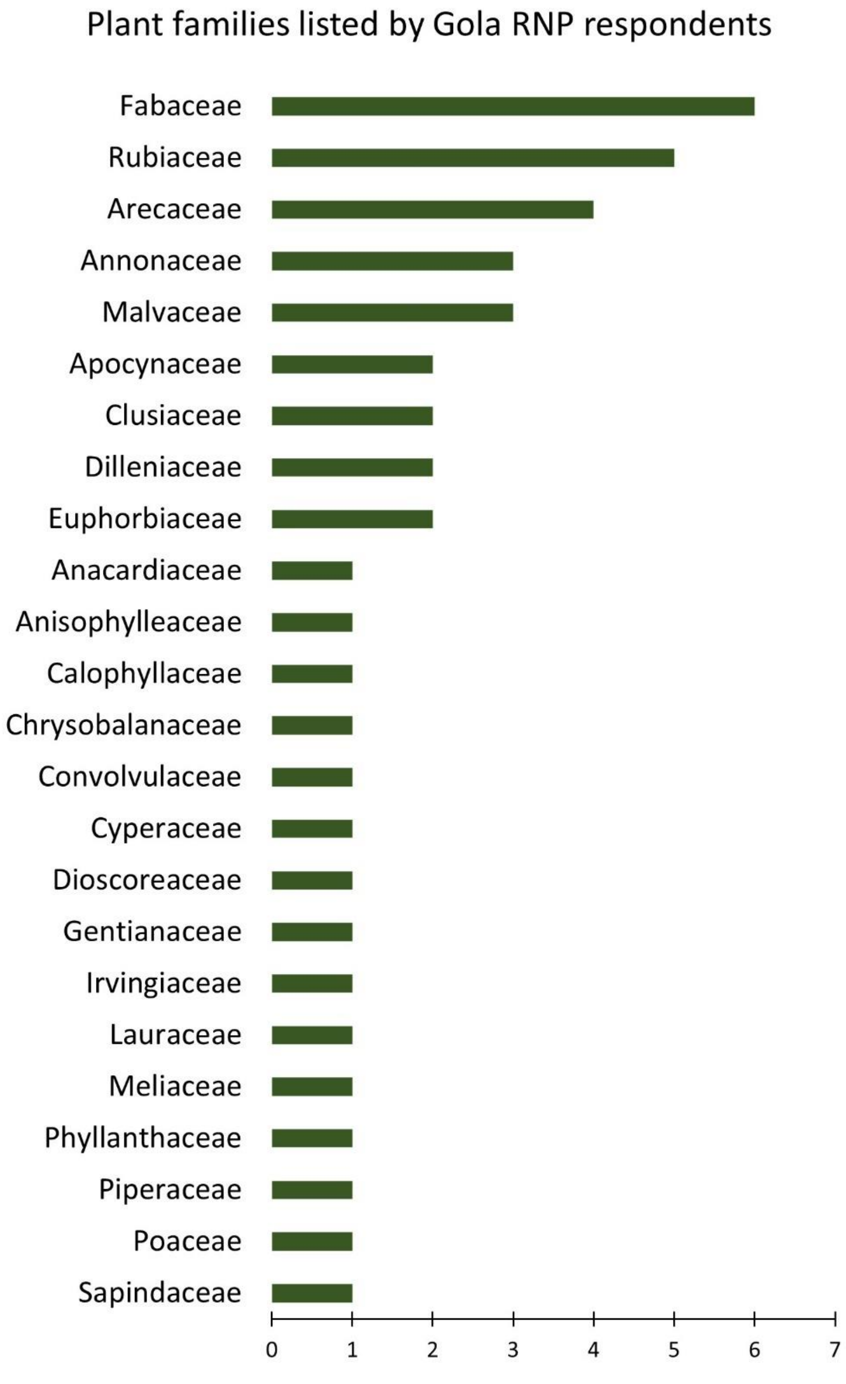


Figure 2.2 Number of plants per plant family, freelisted by all interviewees in Gola RNP. Fabaceae is the plant family with most wild plant species used by people in Gola, and it is also one of the most common plant family within the NP.

Respondents freelisted on average five wild plants (N = 35, mean 4.86±2.84, mode = 6, min = 1, max = 12, variance = 8.07) and 29 respondents reported mainly using plants for traditional medicine, construction, art craft, food, and by-products such as poison powder used in fishing. More than half of the items (55%) were only mentioned once across all interviews, and in five cases, the same respondent listed more than one unique item. Respondent SL47, an adult woman who asks others to collect plants for her and commercialises a portion of the items, listed five unique items out of ten. The respondent who listed the highest total number of items (SL46, 12 items), of which the highest number of unique plants (8 species), was an adult male whose main occupation is a healer. Both respondents are from the same village in Gaura chiefdom, located in Gola central. Women did not cite significantly more plants than men (women: 4.9, men: 4.8, t = 0.104, d.f. = 33, $p > 0.05$), nor did adults in relation to elders (adult: 4.8, elder: 5.0, t = 0.133, d.f. = 33, $p > 0.05$). The type of school attended also did not significantly affect the mean number of plants cited (no schooling = 4.3, official education = 5.3, koranic education = 6.0, $F_{2\text{-}32} = 1.151$, $p > 0.05$).

Wild plants are collected year round according to need (67.7%; e.g. treating an illness, constructing a house). Despite using wild plants for treatment, some respondents mentioned that they *go to the hospital because Gola forest is too far*. When testing for a possible association between distance from the village to the protected forest, and the number of wild plants used, we detected a strongly significant positive correlation (r = 0.456, N = 35, d.f. = 33, p = 0.006), suggesting that 21% ($r^2 = 0.208$) of the variation in average number of wild plants used is explained by variation in distance (Fig. A.2.2 in Appendix 2). This trend was similar but not significant when all 52 respondents answers were included (people who did not use wild plants were assigned a ‘zero’ on the number of wild plants used; Fig. A.2.1 in Appendix 2). However, even though interviews were conducted in both dry and rainy seasons, by chance, all respondents living further than 3 km from the forest boundary were interviewed in the rainy season. Seasonality is a factor known to influence the consumption of plants (Cunningham 2001; Heubach et al. 2011; Kandari et al. 2012; Berihun and Molla 2017; Lepcha et al. 2021) and in this study people interviewed during the rainy season listed nearly twice as many plants than those interviewed during the dry season (W = 62.5, $p < 0.05$). When the same regression analysis was performed on interviews conducted during the dry season only, there was no significant correlation (S = 1774.4, N = 24, $p > 0.05$) (Fig. 2.3).

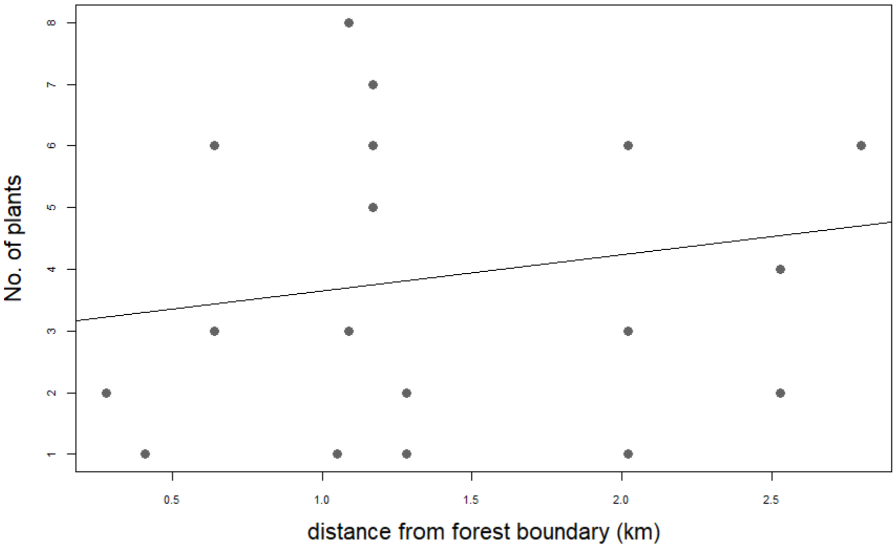


Figure 2.3 Correlation between distance to protected area and wild plant use. Linear regression analysis for correlation between distance from the FEC where the respondent lives to the protected forest boundary, and the number of items freelisted by the interviewee during the dry season. Distance from the village to the forest did not significantly correlate with wild plant use in the region (S = 1774.4, N = 24, $p > 0.05$).

The fact that the villages can be accessed by a driveable road did not influence the number of plants used (no motorable access and motorable access: 4.3, t = -0.943, df = 33 $p > 0.05$), neither did respondent residency (t = 0.084, df = 33, $p > 0.05$), for which the mean number of plants used by people living in Gola central or Gola south was five.

A healthy community forest (human-disturbed forested areas within the 4 km leakage belt; see Chapter 1, section 1.7 for more details) can provide the populations on the forest edge with the most utilised wild plants, as confirmed by 61.5% of the respondents, but some species such as rattan (*Eremospatha macrocarpa* or *Laccosperma secundiflorum*), can only be found in the protected forest (Munro et al. 2013). When asked if they had ever grown commonly used wild plants near their village (within the community forest), three points were raised as to why 20 individuals (62.5%) had not; law enforcements issues:

> *We don't grow NTFPs in the community forest because we are not allowed to collect the young buds from Gola, as we will be arrested. Also, it is hard to find the seeds in the forest because the primates eat them.* [Respondent SL40 - 03.11.2018]

> *The Gola RNP authority hasn't allowed us to collect seeds from Gola RNP and plant in the community forest.*
>
> [Respondent SL51 - 27.11.2018]

ecological reasons (animal conflict with human populations):

> *Because if you bring these plants from Gola and grow them in the community forest, you are inviting the primates to come to the community forest.* [Respondent SL47 - 06.11.2018]

and practicalities:

> *Because we need advice on how to grow these plants.*
> [Respondent SL15 - 10.06.2018]

> *We don't have the time to grow these NTFPs in our community forest because we are too busy with our bush* [garden].
> [Respondent SL43 - 03.11.2018]

Nevertheless, twelve respondents have tried to grow wild plants in their gardens, with limited success. Some people claimed that *plants don't do well due to the nature of the soil* (in the community forest), or *the sun is too strong* for plants to grow, that *it is easy to grow but hard to maintain* wild plants, or that *sometimes they* (wild plants) *are destroyed by animals*. One couple though, had a very positive experience:

> *We have started to plant some of the medicine* [plants] *in our gardens and it is growing now. She* [the wife] *took the seeds from Gola about 15yrs ago, which took a long time to germinate because the soil is not the same as Gola. But the trees are large at the moment.* [Respondent SL42 - 03.11.2018]

Salience of wild plants

Respondents in Gola RNP (N = 35) listed 49 wild plants and a group of six items was identified in Salience analysis as being most important for the sampled population in Sierra Leone (top plants in Table 2.4 from *Garcinia kola* to *Carapa procera*). The cut off for the most salient items (Smith's S (S)) was at least 25% of respondents listing that item.

Table 2.4 Plant salience for human communities in Gola RNP. The table shows the 12 most important (salience score) wild plants used by people in interviewed FECs, its common and English names. For a complete list of plants cited in interviews, plant names in other local languages, the life form (type of plant) and conservation status of each plant can be found in Table A.2.3 in the Appendix 2.

Plant species name	No. times cited[a]	Salience score[b]	Common name (Mende)	English name
Garcinia kola	19	0.388	sagbei	bitter kola
Massularia acuminata	15	0.329	bush ataya	chewing stick tree
Eremospatha spp.	13	0.288	rattan	rattan palm
Piper guineense	13	0.236	mbahem	West-African black pepper
Cassia sieberiana	10	0.196	gbangbei	West African laburnum
Eremospatha macrocarpa	7	0.160	balui	small rattan palm
Nauclea diderrichii	7	0.143	bundui	brimstone tree
Carapa procera	9	0.134	kowi	African crabwood
Xylopia aethiopica	7	0.118	hewi	Ethiopian pepper
Sarcocephalus latifolius	5	0.077	yumbuyabei	strawberry tree
Garcinia afzelii	5	0.074	de nyani	bitter kola
Pentaclethra macrophylla	4	0.066	faawei	African oil bean

[a] Number of times the plant species were listed across all interviews (N = 35); [b] Salience score (Smith's S, values: 0 least important, 1 most important) was calculated using all items listed (N = 49) by the 35 interviewees who use wild plants. Updated species names were confirmed in *The Plant List* (2013), the common names were given by the respondents and spelling confirmed with local plant lists, and English names were obtained in *JSTOR Global Plants* (2021) and *PROTA4U* interactive web database.

Approximately one third of the plant items (32.6%) were only mentioned by either males or females. The top salient plants for men and women included similar items, though with different rankings. The same result was observed for adults and elder (Table 2.5). Small rattan palm (*Eremospatha macrocarpa)* was the plant with the highest salience for people who sell a portion of the plants, followed by African oil bean (*Pentaclethra macrophylla*) and West-African black pepper (*Piper guineense*). Residents in Gola south listed *Garcinia kola* as the top salient plant, whereas respondents from Gola central had *Massularia acuminata* as the most important species.

Table 2.5 Plant salience for each gender and age cohort in Gola RNP. Only the top 10 plants are represented in this table.

	Gender		Age	
Plant species	Men	Women	Adults	Elders
Carapa procera	9 S = 0.11	3 S = 0.18	7 S = 0.15	10 S = 0.08
Cassia sieberiana	5 S = 0.22	6 S = 0.16	6 S = 0.15	3 S = 0.40
Eremospatha macrocarpa	6 S = 0.17	7 S = 0.16	5 S = 0.16	8 S = 0.15
Eremospatha spp.	2 S = 0.36	5 S = 0.17	4 S = 0.25	2 S = 0.50
Garcinia afzelii	12 S = 0.09	16 S = 0.05	15 S = 0.04	4 S = 0.23
Garcinia kola	1 S = 0.39	2 S = 0.39	2 S = 0.35	1 S = 0.56
Irvingia gabonensis	NA	4 S = 0.17	11 S = 0.08	NA
Massularia acuminata	4 S = 0.24	1 S = 0.48	1 S = 0.36	6 S = 0.17
Nauclea diderrichii	7 S = 0.16	10 S = 0.12	9 S = 0.13	5 S = 0.21
Piper guineense	3 S = 0.29	8 S = 0.15	3 S = 0.25	7 S = 0.15

Single values are the salience rank of the plant species for a specific group, S is the item salience score calculated in AnthroTools package (Jamieson-Lane and Purzycki 2016) in R3.6.(*R Core Team* 2022) and 'NA' the item was not mentioned by any respondent within the category.

2.4.2 Cantanhez National Park

Wild plant use

Every interviewee in Cantanhez NP used wild plants that were collected either by the respondent him/herself (94.6%) or someone else (5.4%). On average, people freelisted eight wild plants (mean = 7.96, SD = 3.4, min = 2, max = 18, N = 56, variance = 11.64) when asked to name which they use and that are specifically harvested in the protected area, not from their gardens. Demographic factors such as gender (W = 302), type of school attended ($X^2_2 = 3.95$), occupation (W = 96), and religion ($F_{3\text{-}52} = 2.091$) did not significantly influence the number of wild plants people use (all $p > 0.05$). However, the number of plants used varied significantly across the three age cohorts ($X^2 = 9.67$, N = 56, d.f. = 2, $p < 0.01$). *Post-hoc* comparisons (Tukey test) showed significant differences between elders and the other two age cohorts. A higher number of plants was listed by elders, who used on average 2.5 more plants than adults ($p < 0.05$) and 3.6 than young people ($p < 0.01$). The same analysis was performed for the community groups and I detected significant differences among five of them ($X^2 = 12.56$, N = 51, d.f. = 4, $p < 0.05$). The Biafada, Bijagó and Mandinga community groups were excluded from the analysis due to small number of respondents (2, 1 and 2, respectively). The post-hoc Tukey test showed that people who identified as Fula, listed a higher number of plants than two other communities. Fula people used on average 4.71 more plants than Balanta ($p < 0.05$), and 4.28 more plants than Nalú ($p < 0.05$).

In total, 120 items were inventoried of which 82, belonging to 30 plant families and 72 genera, were identified to species level. The most frequently mentioned plants belonged to the Fabaceae and Apocynaceae families (18 and 9 species, respectively, Fig. 2.4). In the population of Cantanhez, 54% of the items listed (N = 65 items) were only mentioned once. These unique items were listed by 29 respondents from all backgrounds. Out of the 82 plant species recorded, 34% were unique, while for the unidentified plants, all except one ("*acôpa*", listed twice) were unique plants (N = 37). All respondents use wild plants for domestic purposes, but 23.2% also sell part of it. Those who commercialise plants mentioned on average nine wild plants while the rest of the respondents listed 7.6, but the difference was not significant (W = 214.5, N = 56, $p > 0.05$).

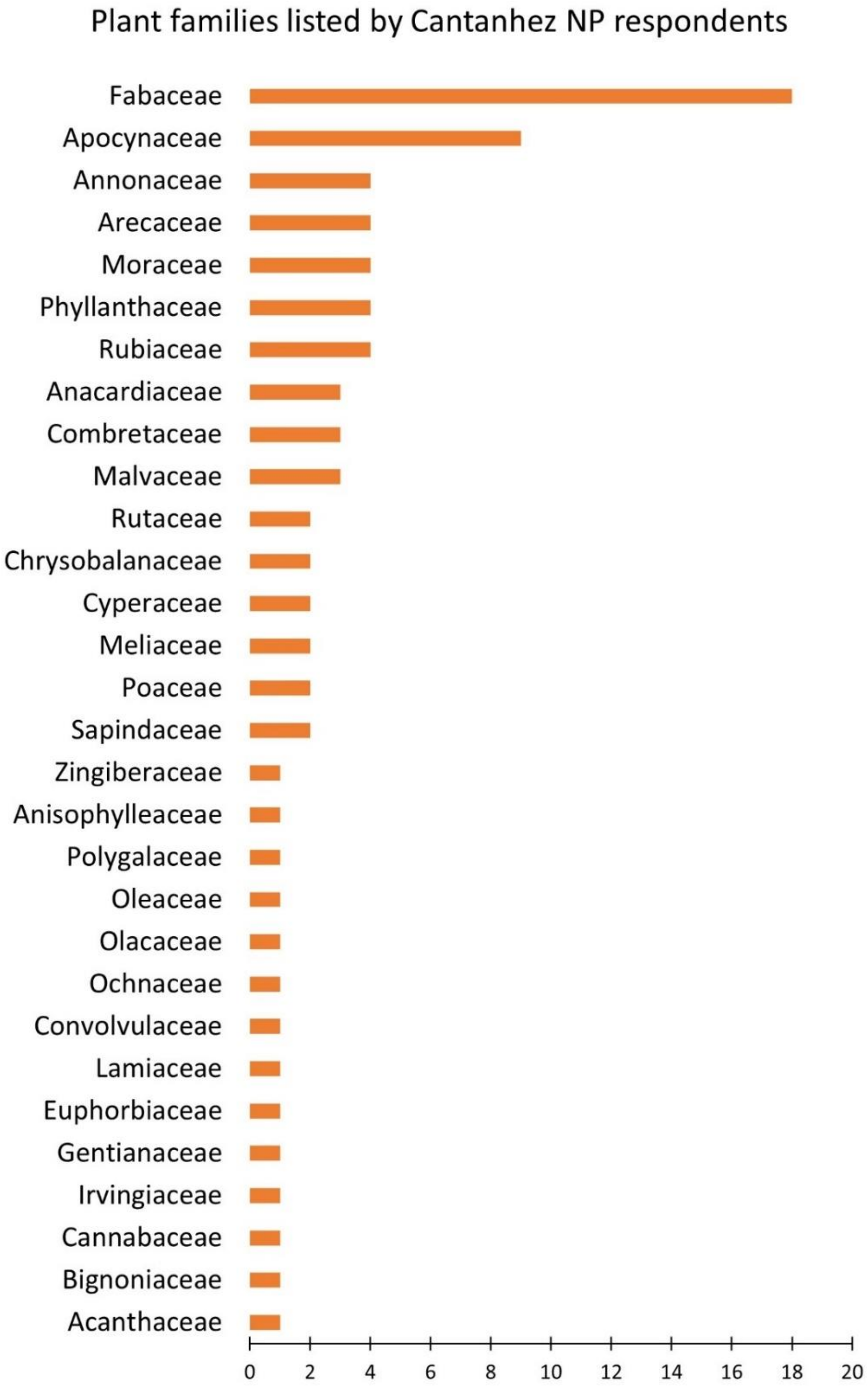


Figure 2.4 Number of plants per plant family, freelisted by all interviewees in Cantanhez NP. Fabaceae was the plant family with most wild plant species used by people in Cantanhez, and this plant family is the most diverse in the park with 132 different species recorded.

We did not ask for specific use of each wild plant. However, 33 of 56 respondents provided this information, enabling us to infer the most common uses: treatments in traditional medicine, food, construction, boat building, fishing and farming tools, and furniture. Other less common uses were also mentioned, like art craft and fuel. The time of the year that people harvest wild plants varied. The dry season was the preferred time of collection (40.4%), while 26.9% collected wild plants when in need (usually associated with illness) and 19.2% all year round. The rainy season was the least mentioned time (13.5%). The youngest town chief interviewed provided details on the plants he collects and when, and their uses:

> *I use wild plants to make the house, canoe with Kapok tree, and stool. The fruit, I pick it to eat between April and May, for example, the velvet tamarind, African locust bean, monkey apple, and I also use it for treatment* [in traditional medicine].
>
> [Respondent GB27 – 05.04.2019]

People from villages in Cantanhez NP harvest wild plants from most terrains in the park, listing primary and secondary forest, the savannah, close to rice plains and freshwater lagoons, and salty mangroves. Two respondents did not know if there were places in the forest where it was forbidden to harvest wild plants, 26 people said they could collect from anywhere, but 28 respondents told us there were some areas of the forest where it is forbidden to carry out such a practice. No differences in the number of wild plants used were found between the two main groups ($W = 258.5$, $N = 54$, $p > 0.05$). These forbidden areas are protected or sacred, and usually under the management of local ritual authorities. However, many of the respondents who said there was not a “forbidden forest”, mentioned that there were some restrictions in some localities. One person explained:

> *The forest is not forbidden if the plants are for traditional medicine purposes, but there is a forbidden forest in case the plants are to be sold.* [Respondent GB2 – 24.03.2019]

and another informed that:

> *There is no forbidden forest, but you have to let the forest rangers,*
> *or the park collaborators know before you go and collect,*
> *especially for African palmyra palm.*
>
> [Respondent GB3 – 24.03.2019]

Variation in the knowledge of a "forbidden forest" was not statistically associated with gender, age, schooling, occupation, community group or commercialisation of plants (all $p > 0.05$).

When asked if it was possible to find preferred wild plants in every part of the protected forest, 10.7% said no or that only some plants were present everywhere. Therefore, we asked interviewees if they had ever tried to grow the wild plants collected in the forest in their gardens as a way to facilitate access to this natural resource. Many had never planted wild plants because *the plants already exist in the garden ('orta') and we maintain those*, or *we are already very busy with our farming and other activities* so, have no time to plant other items, but most mentioned that *there are plenty of wild plants in the forest that is of easy access, so there is no need to plant in the garden ('orta')*. Nevertheless, almost one fifth of the respondents had attempted to grow some wild plants in their gardens but they did not list fewer plants than those who did not attempt ($W = 141.5$, $N = 55$, $p > 0.05$). Out of the ten people, only two were not successful in growing wild plants. The following are some of the quotes reporting successful stories (either direct transcripts or translations from the guides):

> *I planted Guinea pepper, African crabwood, monkey apple, goiaba de lala (Schrebera arborea) around 15-20 years ago. Now, the trees are good. The knowledge was passed to the family. I went to visit my cousin and saw him planting African crabwood in his garden, and that is how I learnt how to do it.* [Respondent GB25 – 04.04.2019]

> *The plant grew well (for example African crabwood), but the rough-skinned plum didn't grow. I tried to plant these trees in dividing border of my garden and someone else.*
>
> [Respondent GB26 – 04.04.2019]

> *I went to get the seeds of African crabwood from Cadique, grew the seedling in a nursery and then planted in the garden. It grows well. If there is a* [wild] *plant already in the garden, I clean the ground around them, so they grow and then I can use for treatment. There are many wild plants in the forest, so I don't need to plant in the garden.*
>
> [Respondent GB27 – 05.04.2019]

> *Oil palm and dry-zone mahogany, I grow seedlings in nurseries and then plant. The other plants I don't grow because I have no interest, it is not very explored, I do not use it much and there is plenty*
>
> [Respondent GB39 – 06.04.2019]

Salience of wild plants

Interviewees across 12 villages in Cantanhez NP listed a total of 120 items, of which 82 were identified to species level. The most salient plants mentioned by at least 25% of the respondents (N = 56) were *Dialium guineense* to *Pterocarpus erinaceus* in Table 2.6 (seven items).

Table 2.6 Plant salience for human communities in Cantanhez NP. The table shows the 12 most important (salience score) wild plants used by people in interviewed villages, its common and English names. For a complete list of plants cited in interviews, plant names in other local languages, the life form (type of plant) and conservation status of each plant can be found in Table A.2.4 in the Appendix 2.

Species name	No. times cited[a]	Salience score[b]	Common name (crioulo)	English name
Dialium guineense	33	0.361	veludo	Sierra-Leone tamarind
Anisophyllea laurina	28	0.348	miséria	monkey apple
Parinari excelsa	26	0.324	manpatace	rough-skinned plum
Cassia sieberiana	23	0.255	canafistra	West African laburnum
Elaeis guineensis	24	0.254	palmera	oil palm
Uvaria chamae	15	0.194	banana-de-santcho	finger-root
Pterocarpus erinaceus	15	0.158	pó-sangue	African rosewood
Khaya senegalensis	10	0.113	bissilon	dry-zone mahogany
Spondias mombin	11	0.101	mandiple	yellow mombin
Sarcocephalus latifolius	11	0.100	madronho	African peach
Avicennia germinans	10	0.093	tarrafe	white mangrove
Landolphia spp.	8	0.083	fole	-

[a] Number of times the plant species were listed across all interviews (N = 56); [b] Salience score (Smith's S, values: 0 least salient, 1 most salient) was calculated using all items listed (N = 120) by the 56 interviewees who used wild plants. Updated species names were confirmed in *The Plant List* (2013), the common names were given by the respondents and spelling confirmed with local plant lists, and English names were obtained in *JSTOR Global Plants* (2021) and *PROTA4U* interactive webdatabase.

Plant salience varied among the demographic groups in Cantanhez NP (detailed in Table 2.7). In some cases, group salience was similar to the whole population salience, but for other groups, plant importance varied (e.g. *Uvaria chamae* is the most important plant for elders, but not for adults or young). All healers were elder, thus the most salient plant for those who frequently use plants as a treatment was the same in both groups (*Uvaria chamae*). Since most respondents were farmers, the top three plants for this group are the same as those in Table 2.6. The most salient plant across Cantanhez NP, *Anisophyllea laurina*, was ranked very low on the healers list (rank 49, S = 0.020).

Table 2.7 Plant salience for each gender and age cohort in Cantanhez NP. Only the top 10 plants are represented in this table.

	Gender		Age cohort		
Plant species	Men	Women	Young	Adult	Elder
Anisophyllea laurina	1 S = 0.38	4 S = 0.29	2 S = 0.44	1 S = 0.43	5 S = 0.23
Cassia siberiana	5 S = 0.20	3 S = 0.35	7 S = 0.14	4 S = 0.31	4 S = 0.26
Dialium guineense	2 S = 0.37	2 S = 0.35	3 S = 0.39	3 S = 0.39	2 S = 0.32
Elaeis guineensis	3 S = 0.28	5 S = 0.21	5 S = 0.22	5 S = 0.22	3 S = 0.30
Parinari excelsa	7 S = 0.19	1 S = 0.56	1 S = 0.49	2 S = 0.39	7 S = 0.18
Pterocarpus erinaceus	4 S = 0.22	17 S = 0.06	4 S = 0.26	7 S = 0.17	17 S = 0.10
Uvaria chamae	6 S = 0.19	6 S = 0.17	8 S = 0.13	12 S = 0.09	1 S = 0.33

Single values are the salience rank of the plant species for a specific group, S is the item salience score calculated in AnthroTools package (Jamieson-Lane and Purzycki 2016) in R3.6.(*R Core Team* 2022).

Salience lists for Fula, Nalu and Sosso were the most similar amongst the five community groups analysed. The tanda community shared many of those same plants but respondents from the Balanta group identified different items as the most salient. For Balanta, African rosewood (*Pterocarpus erinaceus*) is the most salient plant, oil palm (*Elaeis guineensis*) is second, and the white mangrove (*Avicennia germinans*) third. For respondents who sell a portion of the collected plants (N = 13), the most salient species are different from other respondents; *Uvaria chamae*, *Sarcocephalus latifolius* and *Cassia sieberiana* are the most important.

2.5 Discussion

The rapid surveys conducted in this study addressed people who use wild plants collected in two protected forests of Sierra Leone and Guinea-Bissau. These are some of the habitats that support botanic resources used both by humans and primates. Previous studies in Sierra Leone showed that many of the plants used were commonly harvested in farm bush and other communal areas in and around rural villages (Leach 1994; Wollenberg et al. 1998; Munro et al. 2013). In Guinea-Bissau people also collect many botanic items in easy access sites, such as home gardens and orchards (Frazão-Moreira 2009; Catarino et al. 2019). However, the answers obtained from our interviews reveal that a high percentage of people harvest NTFPs from the mature protected forests.

2.5.1 Reliance on wild plants

In Cantanhez NP, every interviewee used wild plants, regardless of gender, age, village, education, community group or occupation. In Gola RNP, as expected, a smaller but still relatively high percentage of users was registered (67.3%). Village location in Cantanhez NP could explain why all people use wild plants. Settlements are in the protected area, which may facilitate the access. In Gola RNP, villages are on the outskirts of the park and people must travel on average 1.9 km to reach the edge of the protected forest (data from current study). But in fact, this factor does not appear to have a direct effect as all respondents in Gola south used wild plants regardless of distance to the protected area (Fig. A.2.1 in Appendix 2).

Although this investigation focused on plant use by humans in two countries with different levels of forest utilisation in each park, some plants were used by both populations (Table 2.8). This is likely because both forests are classified as Upper Guinean Forest and around 260 vascular plant species were recorded in common across both sites. Four of the 13 common plant species had similar salience ranks and served the same purposes in both parks: *Anisophyllea laurina*, *Cassia sieberiana*, *Sarcocephalus latifolius* and *Sterculia tragacantha*. Plants used for fuel wood were not commonly listed in both NPs, as expected in the Guineo-Congolian region (Dounias et al. 2000).

Table 2.8 Wild plant overlap across NPs. Number of items identified to the family, genus and species level enumerated by the human populations of each national park, and those common across the two NPs.

Wild plants listed and identified	Overlap between NPs	Gola RNP	Cantanhez NP
Family	18	24	30
Genus	16	39	72
Species	13	44	82

2.5.2 Spatial variation of wild plants

The difference in choice in the Gola RNP population could be explained by the location of the village of residency (Gola south or Gola central) and the perception of right of use of the protected area. It could be expected that in less isolated places, with better access to modern infrastructures and goods, people would rely less on forest products, particularly medicinal NTFPs. However, this was not the case in Gola RNP, as every interviewee in the wealthier chiefdom in the region (Makpele chiefdom, in Gola south) used wild plants. This may be due to the fact that "modernisation" levels do not have an impact in populations which are among the poorest households in the world (43% live on US$1.90 per day per capita, (World Bank 2020) and depend highly on NTFPs for their livelihoods (Wickens 1991; Angelsen et al. 2014). Activities, such as hunting, mining or logging, are not permitted inside the protected area, but wild plant harvesting is allowed. Yet, our data demonstrates that people are either misinformed about their right of use of the forest, or reluctant to disclose that they collect wild plants if they also engage in illegal activities.

One aspect of Gola RNP that may contribute to fewer people using wild plants than in Cantanhez NP, is the presence of a community forest surrounding the villages. This forest is adjacent to the mature protected forest, has the propensity of being a more productive source of items used by humans, and is likely to have high biodiversity (Leach 1994; Wollenberg et al. 1998; Bulte et al. 2013). This buffer area may serve as a pseudo-protected forest that provides people with many of the desired plant products. The quality of the forest surrounding each village was not assessed, but some respondents mentioned that many of the products could be found there. The questions in the interview addressed specifically wild plants collected in the protected mature forest (high forest – *ngola* in Mende), and in Gola RNP most harvesting takes place in secondary forest and bush fallow areas (Leach 1994; Munro et al. 2013), which could result in the lower percentage of users. Nevertheless, when comparing the two national parks, the possibility that differences observed are a result of cultural factors cannot be discarded.

Respondents in Cantanhez NP listed more than twice the number of plants items (120 items, 82 identified plant species) than people in Gola RNP (49 items, 44 identified plant species), despite the fact that the latest vegetation surveys recorded a similar number of vascular plant species in both national parks: 899 species in Gola RNP (Klop et al. 2008) and 863 in Cantanhez NP (Catarino and Palminha 2018). These numbers are comparable to other research conducted in the NPs. A recent study on the role of NTFPs in Gola forest and Tiwai Island (southwest region adjacent to Gola RNP) registered 39 plant species (Munro et al. 2013). In Cantanhez NP, Hockings and collaborators (2020) studied two Nalú villages and one Balanta and obtained a list of 129 plant species. Both studies accounted for plants collected in any habitat, including farm bush and home gardens. Ethnobotanical research developed in Liberia, with a similar forest composition to the two NPs in this study, registered a higher number of plants among villagers living on the outskirts of Sapo National Park (228 plant species) (Marshall and Hawthorne 2012). Differences in methodology, sample sizes and study site location must be considered when comparing results, but the difference in the total number of items listed in our case studies appears to be an effect of cultural variation between the sites. To support this suggestion, the average number of items listed per respondent did not differ greatly between Cantanhez NP (7.96±3.4) and Gola RNP (4.86±2.8).

2.5.3 Wild plant use in Gola RNP

Differences in the mean number of plants used were observed within the region of Gola but none were statistically significant. Although in Gola population, the tasks performed by men force them to travel further and spend more time in the forest than women (Leach 1994; Munro et al. 2013), men did not list significantly more items than women. Nor did elders in comparison to adults. In many rural communities of Sierra Leone, attending official schools requires travelling to distant urban centres, with limited and costly transportation, and living in places with more amenities (Leach 1994; Bulte et al. 2013). Works conducted in central and south America, concluded that people who studied in urban places, tended to choose biomedicine over traditional medicine (Zent 2001; Quinlan and Quinlan 2007) and consequently have reduced knowledge about traditional medicine. On the other hand, NTFPs may provide a valuable ecosystem service for the poorest and oldest in communities (Sills et al. 2011), increasing their knowledge and use of such products. Thus, we expected to find differences in the number of plants listed between respondents with different types of education, but these were subtle and non-significant. The number of interviewees in this study who practise traditional medicine or sell wild plants was reduced. This could explain why the differences in the number of plants used by these groups were non-significant.

Two of the results did not support the hypotheses that fewer wild plants would be used by Gola south residents and respondents living further away from the protected forest. Villages in Makpele chiefdom have more amenities (e.g. electricity and toilet facilities) than those in other chiefdoms. This is probably due to their being located in a region of high diamond mining activity close to the Liberian border and crossed by the Monrovia-Freetown road, which enhances cross-border trade (Bulte et al 2013). As a result, residents in Gola south were expected to be less dependent on NTFPs and list fewer items in their interviews than those from Gola central. However, the statistical tests did not show significant differences. Additionally, due to the long distance between some villages and the mature forest, a negative correlation was expected with increased distance to the forest. However, no correlation was detected suggesting that people will travel whenever they need a wild plant from the forest, regardless of travel distance. Further research should be conducted on this aspect, as distance to travel might be different for different age or gender groups. Moreover, although the villages visited in this study are at different distances from protected forests, all are Forest Edge Communities (FEC) within a 4 km wide leakage belt around the protected forest. In previous studies, it was reported that residents within the buffer area frequently use forest resources (Bulte et al. 2013; Jusu and Sanchez 2013; Munro et al. 2013), so maybe differences will be detected beyond this limit.

Plant salience varied across the different groups, but most top plants were those described as important plants across Sierra Leone (Munro et al. 2013). These plants (Table 2.4) are used for several purposes, particularly medicine, materials for construction and food, and its commercialisation is limited, as expected (Leach 1994; Munro et al. 2013). For men and the few people who sold plants items, *Eremospatha* spp. which is usually harvested by men and sometimes sold in markets (Munro et al. 2013), was of high importance. This may be linked to its use as ropes in construction, and to produce baskets and mats and other agricultural and household items.

2.5.4 Wild plant use in Cantanhez NP

The mean number of plants used by local people was influenced by social age and community group, but not affected by other demographic characteristics (gender, religion, occupation, education). Despite different activities undertaken by men and women, most are carried out in the forest or areas surrounding the villages. Consequently, there was no significant difference between the number of plants listed by each gender. In Cantanhez NP, both official and Koranic education is available. Furthermore, considerable enhancement of social and cultural knowledge (including natural resources) is obtained through initiation rituals until quite recently practised in the region (Frazão-Moreira 2009). As expected, people who attended Koranic school listed more plants than other groups, but the difference observed was not statistically significant. Healers and respondents who claimed to sell a portion of the plants collected provided longer plant item list, but this difference compared with the rest of the population was not significant. Older people generally have more experience of using wild plants for different purposes, and they may have also gained more knowledge about wild plant use from observations and/or oral testimonies (Quinlan and Quinlan 2007; Frazão-Moreira 2009; Constant and Tshisikhawe 2018; Heineberg and Hanazaki 2019). As predicted, elders in Cantanhez NP listed significantly more plants than the other two groups (adults and young), which could indicate knowledge gained through experience.

The variance in plant salience observed in Cantanhez NP is likely related to the distinctive activities or knowledge of wild plants of each demographic group (Table 2.7). The answers from the group of people who provided the general use of wild plants (N = 33) matched the published literature very closely (Catarino et al. 2006; Frazão-Moreira 2009; Catarino et al. 2020), and the plants with higher Salience were multipurpose items with uses in traditional medicine, as food items, in construction, arts and crafts and as fuel. As all healers were elders, the plants with the higher salience score were similar for both elders and healers but these differed to some extent, from the rest of the population of Cantanhez NP (Table 2.7). The exception was *Elaeis guineensis*, the third most salient plant for elders but 28th for healers. This plant is used to produce oil for cooking, wood for construction and palm wine (Catarino et al. 2006). For adults, *Khaya senegalensis* and *Ceiba pentandra* were two important species ranked lower for elders and young, probably because these trees are used for the construction of canoes and boats (Catarino et al. 2006), and stools (respondent SL51). Fula people listed significantly more plant items on average than other communities, but plant salience was similar to that identified by the Nalú and Sosso people, and generally by the whole population of Cantanhez NP (Table 2.6). One plant item that was highly salient for the Balanta (rank 3, S = 0.26) but not for the other groups was *Avicennia germinans*, the white mangrove. This is likely to be because the Balanta people are specialised in the production of "salt-tolerant" rice ("bolanha salgada") that takes place in areas taken over from the mangroves (Frazão-Moreira 2009; Temudo 2009).

2.5.5 Limitations of the study

This chapter is a result of a study carried out alongside other fieldwork to collect biological samples from primates, hence time was a limiting factor to perform a more detailed investigation. We recognise that the sample size (number of interviews) for some groups in both case studies does not reach the minimum required to validate our hypothesis with statistical significance. We did, however, try to standardise the place and time we conducted the interviews, as variation in the setting may influence the answers of the interviewees (Chaves et al. 2019). Another factor that could have influenced people's answers was the use of interpreters when conducting interviews. Despite aiming to be impartial, research and community assistants were known to local people and associated with a governance entity, which could have caused some discomfort in answering truthfully to certain questions.

Furthermore, there are some limitations when using a freelisting approach: a) it is less exhaustive than other techniques; b) familiarity with the domain may restrict the number of items cited by the respondent; c) listing of items may be limited to current everyday experience (e.g. elements used in different seasons, or during famine periods may be excluded) (Quinlan 2005; Newing 2011). Given the chance to apply a two-phase methodology (focal group followed by individual interviews, or repeating interviews), we could have narrowed the domain of "wild plants used by the respondent that were collected in the protected area" to list items specifically used in traditional medicine, construction, etc., to improve the database of items. When

Quinlan (2017) asked people in Dominica to mention bush medicines, some omitted particular ailments probably because a certain illness did not occur to the respondent at the time of the interview. By repeating the interviews, we would also have given the respondents an opportunity to recall other items that they would have not listed in the first interview (a phenomenon called reminiscence) (Brewer 2002). If possible, we would have interviewed the same person at different time points throughout the year, so we would assess more robustly the effect of seasonality (Newing 2011). One other caveat in this research is that we did not confirm with respondents if they knew exactly the boundaries of the protected area in Gola RNP, although we stressed the importance of origin of the plants for this study in the introductory presentation and throughout the interviews.

2.5.6 Conservation considerations

Understanding in detail how conservation management measures such as village location or right of use of the forest and its products, impact the use of NTFPs by human populations in protected areas and surrounding regions, is crucial for the success of conservation programs. Restricting some of the poorest populations in the world from benefitting from NTFPs and ecosystem services for the sake of fauna and flora conservation is unethical, and alternatives must be proposed and applied. An example that appears promising in this study, is the implementation of a rich community forest nearby villages with plants identified as important for people.

This research could propose some trees to be considered in the future, upon planning and designing of reforestation strategies. For example, the most salient plant identified in Gola RNP was *Garcinia kola*, a tree regarded as a wonder plant because every part is used for some purpose (Adesuyi et al. 2012). The wood of this tree in particular, is highly demanded for construction and furniture, having lead a decline of the species population, which conservation status was lastly evaluated in 2004 as ‘Vulnerable’ (IUCN 2022). Its seeds are exceptionally valued in West and central Africa as a stimulant, in traditional medicine and for poverty alleviation among rural people (Moneim and Sulieman 2019). Despite being cultivated, many farmers are discouraged due to its difficulties in germination caused by seed dormancy. However, efforts have been made to find method to overcome this problem, and that can be easily adopted by farmers who usually have low technological requirements (Agyili et al. 2007). Given the current population decline due to deforestation and high rates of forest loss predicted for the region (RSPB 2021), this tree could be a good candidate for reforestation programs in order to maintain the populations of this species and forest cover in Gola RNP, and favour local communities. In Cantanhez NP, the most salient plants were identified as being under the 'Least concerned' conservation status, but if the expansion of cashew plantations increases, particularly with the migration that is experienced in the region, these plants may become 'Vulnerable' in the near future. One plant commonly used by people locally is, however, heavily exploited for its timber and it is since 2017 under the 'Endangered' conservation category. *Pterocarpus erinaceus* (African rosewood, 'pau sangue' in creole) which is used in West Africa for fuel wood, medicinal purposes and as woodworking material, has seen its population decline globally over the last century (IUCN 2022). Even though many countries have restricted international trade of this timber species, populations may still be at risk due to the high demand for firewood and livestock fodder by some of the poorer rural communities. Nevertheless, this tree has a high seedling survival rate, it is easy to cultivate and it can be propagated by cuttings, posing an excellent plant to include in forest regeneration initiatives that could be developed in Cantanhez NP as a way to mitigate deforestation.

Further research is required to disentangle the complex effects of various factors playing a role in ecosystems harbouring traditional and indigenous communities with different levels of forest use, as well as endangered plant and animal species. One area that deserves further attention, is the use and benefits of having a diverse community forest close to settlements that can provide most of the plants people in the region consider important for their livelihoods, but that can also be habitat to biodiversity.

CHAPTER 3. DNA metabarcoding: a new insight into the diet of the Critically Endangered western chimpanzee (*Pan troglodytes verus*)

Western chimpanzee (*Pan troglodytes verus*) adult feeding at the rescue centre of Tacugama Chimpanzee Sanctuary in Freetown, Sierra Leone.

3.1 Abstract

Traditional dietary study methods are important to identify feeding habits and prey choice in ecology. However, these studies are very time consuming and direct observation of the focal species is often difficult and sometimes impossible to conduct in dense habitats, as is the case of tropical forests. Nowadays, DNA-based methods allow for detection of rare prey where observation is difficult or remains in faecal matter are difficult to identify due to low quantity or digestive degradation. In this chapter I analyse the diet of the critically endangered western chimpanzee (*Pan troglodytes verus*) in two national parks with significantly different levels of human pressure. I use DNA metabarcoding on samples extracted from non-invasively collected faeces, amplifying one plant specific marker, the nuclear ribosomal Internal Transcriber Spacer 2. This method has not previously been applied to the diet of western chimpanzees or other apes, so I aimed to establish an innovative approach to dietary studies as a complement to traditional research on these species. DNA metabarcoding is important for non-habituated primates, which are difficult to observe and especially for elusive populations, such as the chimpanzees of Gola Rainforest that are present in low density. The well-studied chimpanzee community of Caiquene-Cadique inhabiting Cantanhez NP was used for comparative purposes of techniques used in dietary research. DNA metabarcoding proved very successful and a high number of plants was detected for populations at both study sites. Nearly half of the plants were identified in one single chimpanzee, indicating that the methodology used is useful at identifying plant taxa rarely eaten by *P. t. verus*. Though chimpanzee dietary diversity in this study was lower than for other populations, a higher number of samples and longer sampling periods would improve the outcomes. The population at Gola RNP, albeit only six samples collected, fed on 10 plant taxa, including one cultivated food. They consumed more plants from the Urticaceae family, not the most common in the park, likely a result of the high consumption of the *Musanga cecropioides* tree fruit. This tree produces fruits for most of the year, and is mostly present in secondary forest, where four of the six

faecal samples were collected. Sample location probably biased the high frequency of the crop *Abelmoschus* spp. in samples. In Cantanhez NP, the number of plants in chimpanzee diet was higher probably due to the higher number of samples. *P. t. verus* consumed 24 taxa from 18 plant families, and more frequently on *Ficus* spp. in both dry and rainy seasons. This genus is not the most abundant plant, but it is present in various habitat types across the park. Three other fruiting trees contributed greatly to chimpanzee diet and explained seasonal variation. They were two wild plants and one crop (mango) and were identified as preferred foods. Chimpanzees included two more cultivated fruits in their diet, which also presented as preferred foods because they were consumed in the dry season or early rainy season, when fruit is more available in the park. The percentage of crop feeding was similar to what was previously detected for this primate in this park, and data supports the idea that crops are preferred foods for this population. Overall, chimpanzees in Cantanhez National Park appear to have dietary flexibility, but results should be interpreted with care due to the small number of samples obtained in Gola Rainforest National Park. Results from this research contribute to a better understanding of the dietary requirements of this species and may be used in evidence-based primate conservation strategies in the two National Parks.

3.2 Introduction

3.2.1 Background to the study

The importance of diet in species' biology influences the survival, growth and reproduction of individuals, relating to population and social dynamics and species habitat requirements (Robbins and Hohmann 2006). Field research on the diet of apes and other primates has been carried out in depth for the last sixty years. Most studies have used traditional methods such as direct observations, and examination of hard parts, fibres and other material found in faecal matter and stomach contents.

Despite being established practices for dietary assessments, morphological methods require skilled researchers for the accurate identification of partially degraded food items. Errors in this technique may in turn result in taxonomic errors and under- or over-estimated dietary diversity (Symondson 2002). Newer laboratory techniques, such as DNA metabarcoding now provide valuable tools to analyse the diet of animals at a finer scale (King et al. 2008; Pompanon et al. 2012). The earliest study using this molecular technique to study primate diet was applied to lemurs (Quéméré et al. 2013) and since then, at least five studies have been carried out on old and new world monkeys. However, to my knowledge, no study has used DNA metabarcoding to improve the knowledge on the diet of apes, including the critically endangered *Pan troglodytes verus*.

3.2.2 Primate diet: evolutionary relevance and variation

Diet is one of the most crucial elements in the survival of individuals as they rely on the nutrients in food items to grow and reproduce. In evolutionary terms, diet plays a major role in determining body size, gut morphology, and to some extent behaviour and social composition (Campbell et al. 1999; Marshall and Wrangham 2007; Gussekloo 2008). When studying patterns of female aggression in several primate species, Isbell (1991) suggested that in times of food shortage, female aggression was mainly directed at females from other groups except when food was clumped. Then, within group competition would increase. Food resource availability can influence species density and group size, habitat utilisation and activity patterns. For example, in times of low food availability, chimpanzee communities in Tai NP, Côte d’Ivoire, had shorter daily travel distances. When ripe fruit was highly available, their territory expanded presumably because more time could be invested in territorial defence (Herbinger et al. 2001). Sometimes, the availability of specific food types consumed mostly during periods when preferred food is scarce, can have a great impact on species. Population density in gorillas was more strongly correlated to the abundance of herbaceous vegetation, their staple foods, than to other factors (Rogers et al. 2004).

Some organisms have specialized their diet on plants (herbivores), some on animal tissue (carnivores) and others on both food types (omnivores), with several sub-specializations being described with basis on the main food item ingested.

Primate diets are rich in food item diversity, complex in the parts eaten (mostly of plants), and it has proven to be variable over time as a result of food availability in the environment (Robbins and Hohmann 2006). Many primates fall into the omnivore category, with 65% of the 131 species reviewed by Harding (1981) consuming invertebrates and 37% ingesting other animal foods (including eggs). Nonetheless, the most consumed food item among primates is plant-based, including ripe and unripe fruit (90% of the primates selected in Harding's study), followed by 79% feeding on soft plant food (immature leave, buds, shoots, flowers), 69% on mature leaves and 41% on seeds (Oftedal 1991).

3.2.3 DNA metabarcoding in dietary studies

Classical methods used in animal dietary studies rely on direct observations and macro- or microscopic identification of digested food remains from stomach contents or faecal samples. In the case of primates, these methods have been used for decades and the information obtained can provide precise details such as preferred food parts, time of the day that consumption takes place, or the quality and quantity of food ingested (Hohmann et al. 2012). In some cases, data may even unveil unexpected foraging behaviours like hunting for primate meat by chimpanzees (Boesch and Boesch 1989) or using stones to crack the hard shell of nuts (Carvalho 2011). However, primates are mobile, many feed in tree canopies reaching 30-60 metres high, their main food item (fruit) is easily digested, and many plants are only distinguishable by their seeds, which might not be consumed, or leaves, which have lost their physical properties after digestion (Doran-Sheehy et al. 2006). These and other characteristics pose a challenge when trying to identify prey and plant species consumed by primates. Ultimately, traditional methods may not be as accurate or exhaustive as recent DNA metabarcoding techniques.

Over the past ten years, DNA metabarcoding has been successfully used to explore the diet of several taxa, including endangered and elusive species (e.g. carnivores: Shehzad et al. 2012; Jeanniard-du-Dot et al. 2017; herbivores: Ait Baamrane et al. 2012; Buglione et al. 2018; birds: Coghlan et al. 2013; Liu et al. 2018; reptiles: Martin et al. 2021; Tercel et al. 2022). Analysis on the diet of the apex carnivore Australian sea lion revealed a variety of prey being consumed, including two commercially important species (squid and lobster) and two new items (eels and gastropods) (Berry et al. 2017). They further identified primary prey taxa driving dietary variation among the many sampled locations. Seasonal variation directly affects food availability, which reflects on dietary variation. Differences in diet composition of the lowland Tapir population in French Guiana related to fruiting season around the year (Hibert et al. 2013). By using a DNA metabarcoding approach on the elusive rainforest mammal, researchers were able to demonstrate a consistency on the diet characterisation between classical and DNA-based methods, but the latter extended the range of food items and allowed to discover new ones. In primates, at least six studies have used this technique to assess the diet of lemurs, macaques, and old-world monkeys. Three studies have focused on the consumption of fish, insects and other arthropods to provide insight into a more obscure prey item in primates diet, and describe patterns of insectivory (Lyke et al. 2019; Milner et al. 2021; Rowe et al. 2021). The remaining used plant chloroplast markers (*trn*L and *rbc*L) to explore dietary flexibility of golden-crowned sifaka in a disturbed and fragmented habitat of Madagascar (Quéméré et al. 2013), to describe the diet of a small and very elusive population of Indonesian macaques residing only in a State Park (Osman et al. 2020), and to compare method and marker efficiency in wild white-faced capuchins dietary assessment (Mallott et al. 2018).

DNA metabarcoding consists of itemising the DNA present in gut, faecal or environmental samples (e.g. soil, collections of organisms, or air) using a combination of two techniques, DNA based identification and high-throughput sequencing (Deagle et al. 2014). One or more universal DNA barcodes (standardized

DNA regions) are selected according to the organism (or organismal group) of choice, amplified by polymerase chain reaction (PCR), sequenced using next generation sequencing and then compared against a reference database for taxonomic identification (Symondson 2002; Pompanon et al. 2012). Fortunately, the Barcode of Life Database (BOLD) and other research institutions (e.g. NCBI), and industry have invested in DNA metabarcoding and a vast number of libraries are now available (Ratnasingham and Hebert 2007). The number of species and genes studied so far allow for an immense number of combination of sequences yet, in many cases new reference libraries are still required. However, the technique remains prone to errors and biases (Taberlet et al. 2007). Sample collection methods, DNA extraction techniques, barcode markers and bioinformatics choices, and digestion biases may influence the estimation of taxa in diet and erroneously identify species which are not present in the species dietary profiles (Ji et al. 2013; Thomas et al. 2014; Piñol et al. 2019; Schenekar et al. 2020).

3.2.4 The importance of dietary research in primate and forest conservation

Research investigating diet, patterns of habitat use, foraging strategies and the role of primates in the ecosystem, not only contributes to species conservation but also the habitats they occupy. It has become critical to perform further research on nonhuman primates as ~60% of the species are currently threatened with extinction and ~75% have declining populations, a consequence of past and present anthropogenic activities (Estrada et al. 2017). At the same time, forest is currently being lost at an estimated 10 million ha per year, mostly driven by agricultural expansion (FAO 2020). Forests provide ecosystem services such as water retention and carbon sequestration but are also the preferred habitat of many primate populations.

Understanding primate nutritional requirements can aid conservation and management policies that could result in lowering biodiversity decline and speeding population recovery. Chapman and Chapman (2002) proposed the use of selective logging operations or directional felling techniques to protect important tree species for frugivorous or folivorous species, reducing the impact on biodiversity. Studying food acquisition, handling and processing in frugivorous primates which are more disturbance-tolerant, determines their role as seed dispersers and can ultimately contribute to forest regeneration in degraded habitats (Albert et al. 2014; Andresen et al. 2018). The herbivory of some folivorous primates can also play an important role in forest structure and ecosystem maintenance by slowing tree growth and increasing mortality of preferred tree species, creating clear patches for new tree growth (Chapman et al. 2013). Furthermore, folivorous and frugivorous primates are likely to change the spatial distribution of nutrients, as they feed daily on large quantities of food and disperse widely, dropping their faecal matter on the forest floor (Kalbitzer et al. 2019). Lastly, knowledge of the dietary patterns of primates in anthropogenic habitats can identify potential crop damage caused by primate incursions to plantations. Cultivated items provide food and economic stability for many rural human communities, and because these are located in or close to primates territory, conflict between the two parts may arise (Hill 2005; Hockings and McLennan 2012). By understanding better the dynamics in social-ecological systems, it is easier to reduce and in some cases prevent human-wildlife conflict when management strategies are designed to target specific cases (Madden 2004; Robbins and Hohmann 2006; Riley 2007; Hockings and Humle 2009).

3.2.5 The diet of the frugivorous western chimpanzee

Distinct dental characteristics in apes, such as broader incisors and more generalised molars, support a frugivorous diet (Fleagle 2013). Even though chimpanzees are omnivorous and known to be opportunistic feeders, they are the most frugivorous of all apes and ripe fruit accounts for a high proportion of their diet (Wrangham 1977; Tutin *et al.* 1997; Yoshikawa and Ogawa 2015; IUCN Red List). Interannual variation in fruit availability results in dietary fluctuations, as chimpanzees prefer ripe to unripe fruit and travel farther to obtain it when available (Doran 1997; Rogers et al. 2004). These apes do not feed on species from the most commonly available trees but rather on particular and often rare species (Wrangham 1977; Basabose 2002). Nevertheless, to compensate for the lack of some nutrients in their diet (fruits are a high energy food source rich in sugars but low in other nutrients) (Milton 1999; Chapman et al. 2012), chimpanzees also feed on leaves, seeds, flowers, pith and underground storage organs, insects and small to medium-sized vertebrates (Nishida and Uehara 1983; Boesch and Boesch 1989; Sugiyama and Koman 1992; Uehara 1997; Pruetz 2006; Dutton and Chapman 2015). In some cases, chimpanzees use tools to obtain some food types, a characteristic specific of this group of primates. Honey dipping, a technique to extract honey from hives using sticks, has been recorded across the entire species range including Cantanhez NP, Guinea-Bissau, one of the study sites in this research (Bessa et al. 2021). At Fongoli, Senegal, chimpanzees hunt *Galago* using a stick. This behaviour was conducted more often by male chimpanzees, but females had a higher prevalence in tool-assisted hunting events (Pruetz et al. 2015).

Overall, chimpanzee diet is composed of high-quality items, with low fibre and tannin content (Wrangham 1977). Chimpanzees, like most primates, have important and preferred food items. When using traditional faecal analysis methods, important foods are those present in more than 10% of all samples, and preferred items are those that increase in frequency of consumption with increased availability in the habitat (McLennan et al. 2020). Furthermore, chimpanzees can rely on staple food (consumed throughout the year regardless of its availability in the wild), or fallback species which are consumed at an inverse proportion to preferred food availability (Marshall et al. 2009). Studies on chimpanzees recorded a wide range of plants being consumed, with a minimum of 32 species found in the diet of groups in Bwindi, Uganda (Stanford and Nkurunungi 2003) and a maximum of 200 plants in the forests of Bossou, Republic of Guinea (Sugiyama and Koman 1992). This disparity is not surprising as chimpanzees are widespread across Africa, but also the number of individuals examined and months of observations have varied considerably. Even though dietary studies on chimpanzees have been carried out in several distinct habitats, the most important fruiting plants identified across studies belong to the Moraceae family, with the majority of fruits being *Ficus* species.

The diet of *Pan troglodytes verus* (western chimpanzee) has been investigated in most countries where the subspecies inhabits, but data are usually restricted to small populations and are not comprehensive. In Sierra Leone for example, there is only one study carried out by Jones and Cave (1960) that observed the dietary habits of *P. t. verus* as complementary information in the context of longevity and dental disease in the wild. This study mentions fruit and leaves as being part of the diet of chimpanzees in the original mature forest ("high-forest") and in the low, dense secondary forest ("farm bush"), and describes specifically the ingestion of the fruit of oil palm (*Elaeis guineensis*), bamboo shoots, wild yams and, grubs and snails.

In Guinea-Bissau, an exhaustive study was conducted by Bessa *et al.* (2015) but it only focused on one of the 12 communities in Cantanhez NP (Bersacola et al. 2021). During nine months of direct observations and analysis of feeding traces and faecal samples, the team recorded a total of 51 wild plant species in chimpanzee diets. At another site in Guinea-Bissau (Lagoas de Cufada Natural Park), the number of plant species was lower (31 identified plants) but samples were only collected over the dry season (Carvalho *et al.* 2015). Other studies have been conducted on the same chimpanzee population in Cantanhez NP but they mainly focused on the presence of crops in the diet (Hockings and McLennan 2012; Hockings and Sousa 2013).

Cultivated foods form items in chimpanzee diets in anthropogenically altered habitats, particularly when their preferred wild fruit is less available (Hockings and Sousa 2013; McLennan 2013). Crops are an efficient foraging item, which are low in toxicity and high in quality (Strum 1994; McLennan and Ganzhorn 2017). In a comprehensive species-wide study, chimpanzees were recorded to consume 36 species of cultivars across tropical Africa (Hockings and McLennan 2012). The crops consisted of cereals, vegetables and melons, fruits and nuts, oilseed crop among other, but the most frequently eaten were corn (*Zea mays*), banana (*Musa* spp.), mango (*Mangifera indica*), papaya (*Carica papaya*), oil palm (*Elaeis guineensis*), cocoa (*Theobroma cacao*) and sugar cane (*Saccharum officinarum*).

3.2.6 Aims and predictions

This will be the first study to use DNA metabarcoding to study the diet of an elusive and unhabituated ape. In this chapter I aim to provide a more complete description of two west African populations of the critically endangered ape *Pan troglodytes verus* living in Gola RNP (Sierra Leone) and in Cantanhez NP (Guinea Bissau). Exploring the dietary composition of western chimpanzees in these two sites with contrasting forest topologies and human-altered landscapes, will strengthen the research by providing comparative data which can be baselined against future studies.

Specific aims:

(a) To characterise the diet of an ape sub-species in a disturbed and in a non-disturbed habitat, using a DNA metabarcoding approach;

(b) To assess intraspecific variation in the diet of western chimpanzees present in Gola Rainforest (Sierra Leone) and Cantanhez (Guinea-Bissau) National Parks;

Predictions:

1. Plant dietary richness estimates will be higher than previously found at other chimpanzee study site as a result of using a molecular approach rather than traditional methodology.
2. A marked seasonal variation in the diet composition of chimpanzees will be observed within each study sites. Due to annual fruit availability, chimpanzees will feed on different species in the dry and in the rainy season.
3. Chimpanzees from Gola RNP will include more diversity of wild foods in their diet than the population from Cantanhez NP, as a result of a larger and better preserved forest in Gola RNP.
4. Agricultural food will be consumed by chimpanzees when other preferred items are not commonly available in the wild. I predict that crops will be present at a higher frequency in the diet of chimpanzees inhabiting the forest-

savanna-mangrove-agricultural mosaic of Cantanhez NP, in comparison with individuals living in the less disturbed forests of Gola RNP.

3.3 Methods

3.3.1 Study sites

The two study sites are located in West Africa. Gola Rainforest National Park in Sierra Leone is around 750 km^2 areas of continuous semi-deciduous rainforest with reduced anthropogenic pressure. Human communities are displaced outside park boundaries and heavy patrol control takes place within the protected area. Biodiversity is high, with ten primate species present in the area, including the western chimpanzee (*P. t. verus*) and Bay colobus (*P. b. badius*) (Lindsell et al. 2011). Gola forest is dominated by trees reaching 40m in height in the primary forest, large areas of secondary forest resultant from historical logging activities prior to the civil war and former farming sites, and forest and herbaceous swamps (Klop et al. 2008; Brncic et al. 2010). Intensive logging took place in Gola South block and the western part of Gola Central. The latest botanical survey in Gola RNP identified 899 plant species, of which 232 are trees (Klop et al. 2008). The most abundant plant families are Fabaceae and Euphorbiaceae, with *Heritiera utilis*, *Brachystegia leonensis* and *Cynometra leonensis* the most common trees (detailed information on forest types and common plants in Table A.1.1 in Appendix 1). The rainy season starts in May and ends in November, with July and August being the hottest months. Young foliage production peaks at the beginning of each season, with flowering occurring only in the early dry season, followed by the fruiting season. Despite there being two foliage peaks, young leaves are present throughout most year due to differences in leaf production from different plant species (Oates 1988).

Cantanhez NP in Guinea-Bissau is a mosaic of forest and agricultural fields, with few conservation measures applied. The park covers an area of ~1,000 km^2 comprising of the last place in the country where sub-humid forest can be found. This type of vegetation is characterised by large trees such as *Ceiba pentandra*, *Parinari excelsa*, *Sterculiaa africana*, *Dialium guineense* and *Ficus* spp., and several liana species like *Hunteria elliotii* and *Xylopia aethiopica* (Scott 1992; Rodrigues et al. unknown). One of the most common naturalised trees in the park is the oil palm (*Elaeis guineensis*), which is exploited by human populations. The most common plantation in the park is the cash crop cashew tree (*Anacardium occidentale*), which density has been increasing rapidly in the past years (Catarino and Palminha 2018). Cantanhez has a marked rainy season between June and November, with the other months being mostly dry. Young foliage has a peak in production in July and August, but new leaves are available annually depending on plant species. Flowering is most common in March, with ripe fruiting happening between February and May (Bessa 2014).

For a more detailed description of both national parks, please refer to Chapter 1, section 1.7, and Table 1.2

3.3.2 Study species

Common chimpanzee (Pan troglodytes (Blumenbach, 1799))

The genus *Pan* (family Hominidae) comprises two species, the common chimpanzee (*Pan troglodytes*) and the bonobo (*Pan paniscus*) (Groves 2001; Fleagle 2013). Common chimpanzees (hereafter chimpanzees) are the most widely distributed of all African apes (Butynski 2003). The species is distributed across the African forest belt, from western Tanzania in the east, to southern Senegal in the west (Fig. 3.1), occupying a range of habitats including dense rainforest, secondary forest, farmlands and bush, woodland savanna and gallery forest (Davies 1987; Grubb 1998; Hohmann et al. 2012). *Pan troglodytes* has been classified into four subspecies, *P. t. schweinfurthii* in the East, *P. t. troglodytes* in central Africa and two subspecies in west Africa, *P. t. ellioti* which is restricted to Nigeria and Cameroon, and *P. t. verus* in Upper Guinean countries (Oates and Nash 2011). All subspecies are classified as

Endangered under the IUCN Red List (2022), except for *P. t. verus* which is Critically Endangered with a decreasing population trend (Humle et al. 2016).

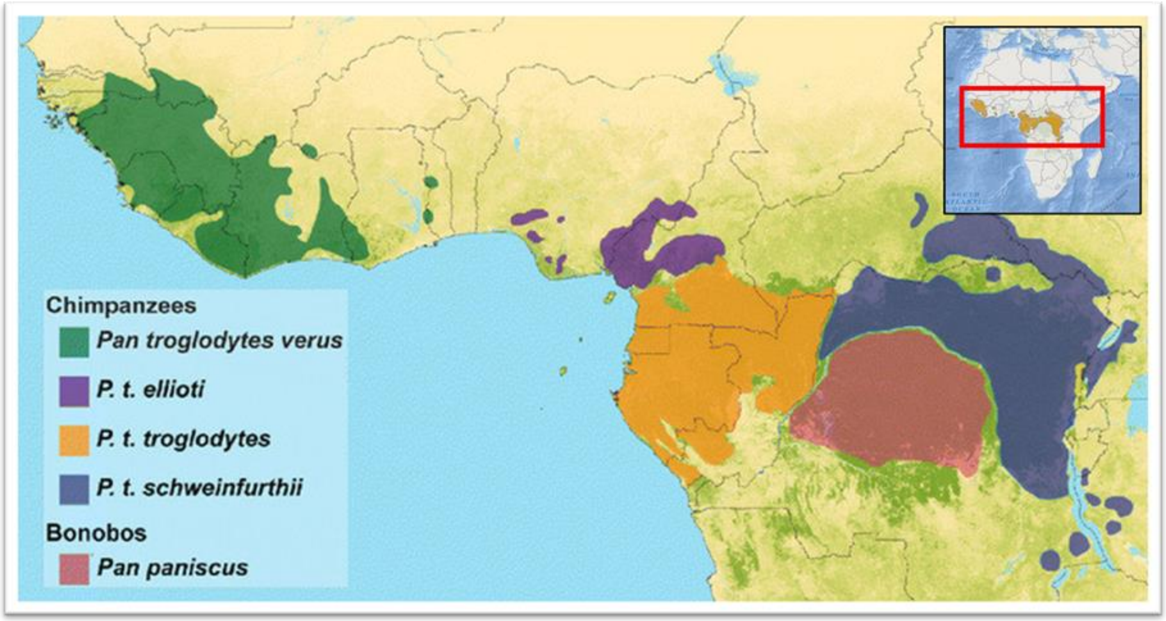


Figure 3.1 Chimpanzee spatial distribution. The map shows the distribution of the *Pan* genus, composed of chimpanzees and bonobos, across Africa. In West Africa, distribution of the critically endangered subspecies of western chimpanzee (*Pan troglodytes verus*) (Sesink Clee et al. 2015).

Chimpanzees are great apes, with males weighing 40-70 kg and females 27-50 kg. Generation time is approximately 25 years (Langergraber et al. 2012). They are highly social, living in communities of several adult males and more females (usually), with a patrilineal society (Goodall 1983; Matsuzawa 2006). Chimpanzee groups can have between 20-150 members (Goodall 1986; Boesch and Boesch-Achermann 2000) and have a fission-fusion social organisation, where their main group subdivides into smaller communities ("parties") over short time periods (e.g. foraging and travelling) (Morin et al. 1993; Chapman 1994; Anderson et al. 2002). This can regulate feeding competition, provide resource exploitation flexibility, and allow males to maximise reproductive female monitoring, but also allow for quick responses to environmental change (Lehmann and Boesch 2004). Chimpanzee community home ranges depend on resource availability such as water and food (Lehmann and Boesch 2003), and can extend between 8 and 89 km^2 (Pruetz 2018; Vieira et al. 2019).

Western chimpanzee populations (*P. t. verus* (Schwarz 1934)) (Fig. 3.2) are currently estimated to number between 17,600 and 96,600 individuals (Heinicke et al. 2019) extending across seven coastal countries (from south Senegal to Ghana) plus southwest Mali. The subspecies has been extirpated from Benin, Burkina-Faso and Togo, and suffered an estimated 80% decline in 24 years (Kühl et al. 2017). Most of the geographic range is unprotected, with 83% living outside high-level protected areas. Consequently, habitat loss and fragmentation have a significant direct negative impact on chimpanzee populations, and genetic and behavioural diversity. Populations become isolated in small habitat fragments, movement is restricted due to lack of suitable habitat for dispersal, impeding gene flow and leading populations to a reduction in genetic diversity (Radespiel and Bruford 2014). Also, wild food resources become depleted and less diverse (IUCN SSC 2020 references there in). Indirectly, habitat reduction or disturbance increase inter-community and human encounters which increases primates stress, exposure to pathogens and changes of being hunted for meat or pet trade (Kormos et al. 2003). Physical encounters with humans in cultivated fields or around settlements can result in retaliatory killings, and movement between forest fragments imply in many cases, crossing roads which can result in vehicle collisions (McLennan and Asiimwe 2016). The Western Chimpanzee Action Plan 2020-2026 suggests undertaking impact assessments, implementing fire management, and defining best practice for mitigating negative impacts of human-chimpanzee interactions, supported by ongoing research to address data gaps. The involvement of regional and international stakeholders is crucial to address the illegal chimpanzee trade, monitor disease transmission, plan land-use, and maintain or establish protected areas.

Figure 3.2 Study species and habitat disturbance in Cantanhez NP. Female individual of *P. t verus* in the forests of Cantanhez NP (left), and slash and burn practice for land conversion into agriculture fields (right).

Pan troglodytes spp. verus (Schwarz, 1934) in Gola Rainforest National Park

In Sierra Leone, the western chimpanzee is found in a wide range of habitats, from savanna to semi-deciduous and tropical forests (Brncic et al. 2010). Nevertheless, despite most individuals living in agricultural and anthropogenic landscapes, Sierra Leone is a stronghold for this subspecies harbouring one of the largest estimated populations with 5,925 individuals (CI: 1,951-12,668) (Heinicke et al. 2019). One of its National Parks, Gola RNP, was chosen as a site enabling chimpanzee persistence due to low degree of forest loss, steep terrain mostly in Gola north, the undertaking of research and its relatively remote location (Heinicke et al. 2019). The population density of *P. t. verus* in Gola RNP in 2009 was 0.27 individuals per km^2 (95% CI = 0.18 – 0.42) with approximately 300 chimpanzees in total (95% CI = 203 – 458) within the project zone (protected and community forest) (Ganas 2009). Densities in the park and across Sierra Leone are low, even in human-dominated landscapes (densities across nine countries ranged from <0.01 to 6.3 individuals/km^2), and lower when compared with areas of similar size (Cavally, Côte d'Ivoire, 791 km^2; Sangaredi, Guinea, 715 km^2) (Kühl et al. 2017; Heinicke et al. 2019). Chimpanzees tend to build their nests in open areas where undergrowth density is not too high, and six of 22 tree species were preferred, accounting for 70% of all nests encountered (e.g. *Heritiera utilis* and *Parinari excelsa*). The preference observed might not be random but instead related to fruit availability (Lindsell and Klop 2013; Barca et al. 2018a) but further research on the subspecies' diet is required. Effective planning and management for protecting the remaining intact forests are critical for the conservation of the remaining chimpanzee populations (Brncic et al. 2010).

Pan troglodytes spp. verus (Schwarz, 1934) in Cantanhez National Park, Guinea-Bissau

The population of the western chimpanzee in Guinea-Bissau was recently estimated at 1,908 individuals (CI: 923-6,121) (IUCN SSC 2020) and although the subspecies is widely distributed across the country (Carvalho et al. 2013; Bersacola et al. 2018), the limits of its distribution are not yet clear. Despite only a few chimpanzee population estimates being nationwide, the abundance for Guinea-Bissau is low when compared with Liberia (7,010) and Sierra Leone (5,580), but nearly twice the estimated population in Guinea (1,100) (Kühl et al. 2017). In Guinea-Bissau, the largest communities of *P. t. verus* are found in three protected areas, including Cantanhez NP. The most current estimate in this NP indicates between 376 and 2,632 chimpanzees (Torres et al. 2010). The habitat in this region is extensively used by humans and chimpanzees, which has led to comprehensive research on the co-use of habitat and co-selection of natural resources by both groups (Hockings et al. 2009; Hockings and McLennan 2012; Catarino et al. 2020; Hockings et al. 2020; Bersacola et al. 2021). The chimpanzees of Guinea-Bissau face threats similar to other regions in Africa. Deforestation due to slash and burn agriculture is a major risk (Fig. 3.2) and, although hunting for bushmeat is not a common practice due to species-specific taboos (Costa et al. 2013; Sousa et al. 2014), individuals are captured for the pet trade (Casanova and Sousa 2007), body parts are used in traditional medicine (Minhós et al. 2013b), and there are occasional fatal encounters with farmers or illegal snares (Gippoliti and Dell'Omo 2003).

3.3.3 Data collection and production

3.3.3.1 Faecal sampling from non-human primates

The methods described in this chapter were standardised as much as possible between study sites. Additionally, because this work is part of a larger project on primate genetics, microbiome and parasitology, field surveys and sample collection were aimed at any primate species, including the three subspecies of focus in this project: *P. t. verus*, *P. b. badius* and *P. b. temminckii*. Fieldwork and laboratory descriptions mostly refer to the primate species included in this study. Statistical analyses are adjusted for each chapter and by corresponding primate species. Export and import permits for the transportation of non-invasive biological samples from endangered primates were obtained from each country involved in the process.

The objective was to collect non-invasive samples (faeces) from red colobus and chimpanzees when walking through the forest in silence and at a relatively slow pace (~2km per hour) to facilitate primate detection. The local guides would detect any movement on the trees or primate calls, identify the primate species and count the individuals, and then confirm that they were feeding. We would halt and wait for about 10-20 minutes before searching for fresh faeces and would then collect it using non-invasive methods. To avoid sampling the same individual, we only collected samples that were two meters apart or more. In some cases, primates were not detected but the team nevertheless searched for primate signs (feeding traces or urine smell).

Between April and June (dry season) and November and December (rainy season) 2018, we surveyed previously determined high primate density areas (Klop et al. 2008; Ganas 2009; Brncic et al. 2010; Barca et al. 2018a), which were some of the most pristine forests in Gola central and south. Gola north was not surveyed due to time and budget limitations. We used line transects established by Gola RNP for research purposes, which meant travelling into the deep forest and camping for a few days at a time. We sampled five transects in Gola south, eight in Gola central (Fig.

3.3) and one site in the community forest where chimpanzees had been observed. Each transect (<4 km) was surveyed twice as we would use the same path to return to the camp site. However, to maximise sampling, we opportunistically collected primate faecal samples whenever we detected individuals visually, acoustically or using other traces. Because both red colobus and chimpanzees are diurnal, the survey hours started at 7.30 a.m. and lasted until the whole transect was completed (sometimes the return to the camp site was around 4 or 5 p.m.). A total of 402 faecal samples were collected in Gola RNP: 284 putative red colobus, 20 putative chimpanzees, and 98 unknown primates. Faecal samples were preserved in HDPE plastic bottles (50ml sterile screw top tubes) containing silica gel to desiccate the sample. This technique allowed us to extract DNA from the host (primates) and from all other organisms in their diet, including plants.

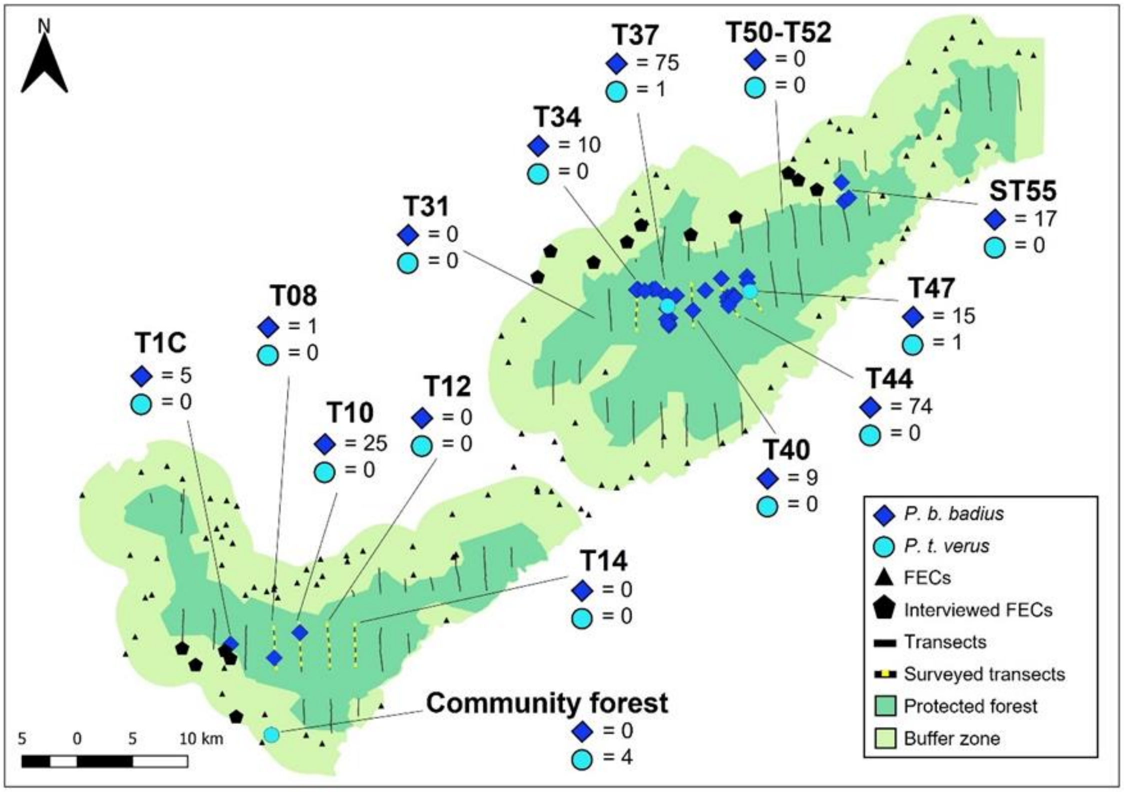


Figure 3.3 Chimpanzee and red colobus sampling in Gola RNP. Faecal sampling in 13 forest transects and on community forest (buffer zone) site in Gola RNP. Sample size refers to samples collected in the field and later molecularly identified in the

laboratory as Bay colobus (*P. b. badius*, N = 231) and western chimpanzee (*P. t. verus*, N = 6). The map also depicts the location of all forest edge communities (FECs) within the community forest and those villages where interviews were conducted. The bottom block of forest is Gola south, where logging was more intense, and the middle block in Gola central.

In 2019, fieldwork took place in Cantanhez NP, Guinea-Bissau between March and April (dry season), and a later survey was conducted by one community researcher during October and November (rainy season). To survey the forest belonging to a village, we asked permission to the village chief and hired a community member to join us for the day. We visited the best-preserved forests in Cantanhez NP known to harbour the two primate species of interest (Minhós 2012; Sá 2013; Bessa 2014) and instead of transects (non-existing in this NP), we travelled through paths used by people to reach the forests. We surveyed 15 forests patches ("matos") plus the forest around the base camp in Jemberém (Fig. 3.4). As in Gola RNP, we surveyed the forest from the moment we left our base camp village (Jemberém).

A total 338 faecal samples were collected in Cantanhez NP:162 putative red colobus, 103 putative chimpanzees, and 73 unknown primates. The same storage method was used for these samples.

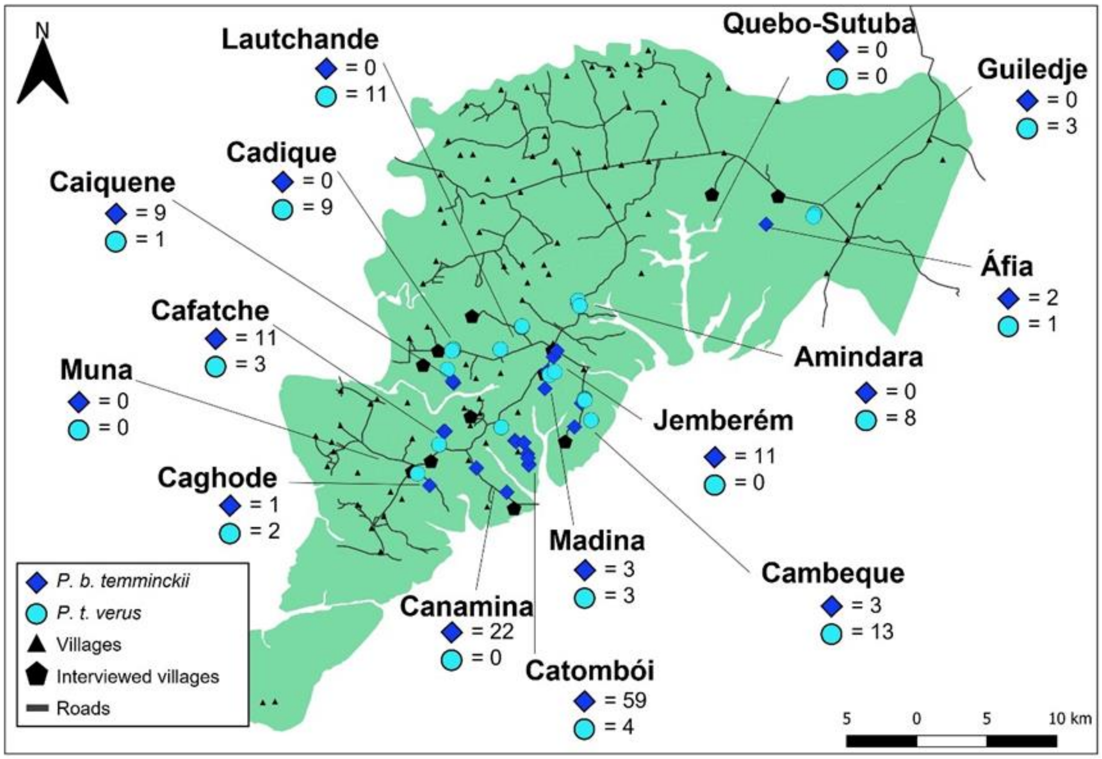


Figure 3.4 Chimpanzee and red colobus sampling in Cantanhez NP. Faecal sampling in 15 forest patches in Cantanhez NP. Sample size refers to samples collected in the field and later molecularly identified in the laboratory as Temminck's red colobus (*P. b. temminckii*, N = 121) and western chimpanzee (*P. t. verus*, N = 58). The map also depicts the location of all villages within the protected area and those villages where interviews were conducted.

3.3.3.2 DNA extraction and primate barcoding

DNA was extracted from 740 faecal samples using a commercial kit for DNA extraction (QIAmp DNA stool kit – Qiagen) with modifications outlined in Doc. A.3.1 in Appendix 3. All extractions were carried out in a laminar flow hood previously cleaned with bleach (20% dilution) and ethanol 70%, and the lab material and hood were exposed to UV radiation for 15 minutes prior to extraction, to avoid human contamination during the process. For each set of samples extracted, a positive and a negative control were added. At the final stage, we measured DNA yield in Nanodrop^{TM} 1000 Spectophotometer including the negative controls to test for human and/or sample cross-contamination (value < 5.0 ng/μL valid extraction). The negative controls were also used at a later stage (section 3.3.4.1). Faecal species identification in the field can be uncertain, especially when primates are not visually detected. Hence, molecular mitochondrial DNA barcoding using species specific or universal markers was used in amplification (see details in Table 3.1). We performed polymerase chain reactions (PCR) in 10μL total volume, with final concentrations of 2.5x of My Taq HS Mix (Bioline®), 1μM of each primer and ~ 30ng of template DNA. We used Cytochrome *b* universal primers for samples of unknown primate species or those that did not amplify with the marker corresponding to the putative primate species.

Table 3.1 Taxonomic identification of faecal samples. Mitochondrial DNA markers were used to perform amplification of the primates’ DNA for species identification of faecal samples. Details on the primers and PCR conditions (temperature, time and cycles) used in this study for different target species.

For putative colobine samples

Region	Hypervariable domain (HVI) of the control region (D-loop) (Minhós et al. 2013a)		
Size	478 bp		
Primer forward	L15449Clbs:	5’CCRCCAATACCCAAAACTGG 3’	
Primer reverse	H115973Clb:	5' TGATCCATCGTGATGTCTTA 3'	
PCR cycle	Temp. (ºC)	Time	Cycles
	94	2 min	
	94	60 s	45x
	63	60 s	
	72	60 s	
	72	10 min	

For putative chimpanzee samples

Region	Hypervariable domain (HVI) of the control region (D-loop) (Sá 2012)		
Size	600 bp		
Primer forward	L15926:	5' TACACTGGTCTTGTAAACC 3'	
Primer reverse	H16555:	5' TGATCCATCGTGATGTCTTA 3'	
PCR cycle	Temp. (ºC)	Time	Cycles
	95	2 min	
	94	30 s	45x
	51	60 s	
	72	60 s	
	72	10 min	

For unknown primate samples

Region	Cytochrome b (Gaubert et al. 2015)		
Size	402 bp		
Primer forward	GVL14724:	5’GATATGAAAAACCATCGTTG 3’	
Primer reverse	H15149:	5’CTCAGAATGATATTTGTCCTCA 3’	
PCR cycle	Temp. (ºC)	Time	Cycles
	94	2 min	
	94	30 s	35x
	50	30 s	
	72	30 s	
	72	15 min	

PCR products were sequenced for the forward primer in an ABI 3130 XL Genetic Analyzer (Applied Biosystems, Foster City, CA) or outsourced to Eurofins Genomics UK Ltd and Macrogen Europe BV. For unsuccessful DNA amplifications, we verified sample DNA concentrations (too low, <50 ng/µL or too high, >1000 ng/µL) and adjusted the initial DNA volume in subsequent PCRs or repeated the sample extraction. Raw sequences were manually checked for accuracy using Geneious 11.0.3 (Kearse et al. 2012) and compared to all primate sequences available on the NCBI database (https://www.ncbi.nlm.nih.gov/) using the Basic Local Alignment Search Tool for nucleotides (BLASTn, Altschul *et al.* 1990). Species identity for 626 samples was confirmed using the BLASTn algorithm on similarity (threshold distance), and 114 samples were rejected due to no amplification or unsure species assignment.

3.3.3.3 Plant metabarcoding and high throughput sequencing

Even though all samples that successfully amplified with mitochondrial primers and identified to a primate species were used in the next steps (N = 626), data presented in this chapter will only refer to chimpanzee samples (*P. t. verus*). At this stage, a total of 64 western chimpanzee DNA samples were selected to be used in the initial phase of the DNA metabarcoding process (amplification with UniPlant markers, below). These referred to samples collected in 2018 in Gola RNP (6 *P.t.verus*), and in 2019 in Cantanhez NP (58 *P.t. verus*).

Plant DNA amplification

Presence of plant DNA in the primate faecal extracts was confirmed with an amplification of the second internal transcriber spacer (ITS2) of nuclear ribosomal DNA using universal plant primers UniPlantF (5’-TGTGAATTGCARRATYCMG-3’) and UniPlantR (5’-CCCGHYTGAYYTGRGG-TCDC-3’) (Moorhouse-Gann et al. 2018). PCRs were carried out in 5µL total volume with final concentrations of 2.5x multiplex master mix and 0.1µL of Q solution (both Qiagen, Manchester, UK), 0.2µM for each primer and ~ 60ng of template DNA. The PCR cycle initiated with a denaturation step of 95ºC for 10 minutes, followed by 40 cycles at 95ºC for 30s, annealing temperature of 56ºC for 30s, and 72ºC for 60s, and a final extension of 10 min at 72ºC (Moorhouse-Gann et al. 2018). In each PCR, a positive control sample of plant DNA to confirm amplification success was added, along with two negative samples (pure primate DNA and nuclease-free water) to control for possible contamination. Amplification success and amplicon size were verified in a 2% agarose gel electrophoresed at 110 Volts for 40 minutes. Samples that failed to amplify at this stage were excluded from the rest of the process (N = 2 *P. t. verus* from Cantanhez NP).

MID-tag Polymerase Chain Reaction

The remaining 62 samples (Gola RNP 2018: *P.t. verus* = 6; Cantanhez NP 2019: *P.t. verus* = 56) were amplified with MID-tag primers, which are UniPlant primers labelled with a unique 10bp molecular identifier tag. Forward and reverse primer combinations allow for identification of each DNA sample in the post-sequencing process. PCRs of 25µL reaction volumes containing final concentrations of 2.5x multiplex master mix and 2.5µL of Q solution (both Qiagen, Manchester, UK), 1.0µM for each primer and ~ 60ng of template DNA. The PCR cycle initiated with a denaturation step of 95ºC for 10 minutes, followed by 40 cycles at 95ºC for 30s, annealing temperature of 56ºC for 30s, and 72ºC for 60s, and a final extension of 10 min at 72ºC (Moorhouse-Gann et al. 2018). Each 96-well PCR plate included 80 primate samples, 12 negative controls, two blank controls and two positive controls. Negative controls were either extraction or initial PCR negatives treated as DNA samples and used to test primer contamination for each MID-tag. Blank samples (PCR mix and nuclease-free water) were placed in unused MID-tag combinations to identify tag-jumping. Positive controls were a mixture of plant DNA non-native to Africa used for amplification success and artefacts. DNA from European plant species was extracted using a salting-out method and prepared in two different concentrations.

High-throughput sequencing

All PCR products were quantified (ng/µL) on a QIAxcel Advanced System (©Qiagen) and negative controls were checked for PCR contamination. If contamination was detected, the PCR was repeated. Sample pooling for each PCR plate was based on equimolar concentrations and final concentration ranged between 0.17 and 0.26 ng/µL. The volume pooled for negative and blank samples was the same as the average volume of all other samples in each plate. Each DNA pool was cleaned using SPRIselect beads (Beckman Coulter, Brea, USA) in a ratio of 1:1.1 to retain fragments between 300 and 500 bp (ITS2 fragment size after MID-tag PCR). The concentration of each pool DNA was determined after clean-up using a Qubit dsDNA high-sensitivity assay kit. Pools were equimolarly pooled into "super-pools" (SP) and DNA concentration was once more measured in the Qubit dsDNA high-sensitivity assay kit. Three SPs containing 256, 267 and 268 samples, including all controls, were achieved with similar DNA concentrations (60.4, 61.8 and 63.0 ng/µL, respectively). Each SP was normalised to 100ng of DNA for adaptor ligation under Illumina sequencing library preparation using a NEXTflex Rapid DNA-Seq Kit (Bioo Scientific, Autin, USA) and AMPure XP Beads clean-up. Each SP was assigned a different Illumina index and was sequenced on a 250bp paired-end reads V2 chip on an Illumina MiSeq.

3.3.4 Data analysis

The bioinformatics and data processing described below were consistent across all three SPs. At a later stage, all samples were put together under the same file to proceed with contamination clean up. Finally, for the statistical analysis, several sub-sets were created in order to group samples according to primate species, NP or year of collection.

3.3.4.1 Bioinformatic analysis and data processing

The bioinformatics pipeline followed the process initially designed by Drake et al. (2022) with modification resulting from updated programs and improved methodology in the taxonomic assignment step. The pipeline was performed under LINUX and comprised several programs and scripts written in BASH and PERL. The Illumina V2 250bp paired-end reads MiSeq chip used to sequence all primate species samples collected across the different NPs and years, generated a total of 12,393,811 reads, with 4 to 5 million reads per SP. Overall truncation of the MID-tag primers did not exceed 9%. This means that 91% of all reads (11,278,368 reads) amplified with the full length of the MID-tag primers (10bp) for both forward and reverse primers. Raw sequences were trimmed, aligned (forward and reverse sequences) and checked for quality of reads using FastP v0.20 (Chen et al. 2018). Reads with low quality score (<Q33; total of 66 reads), which were too short (<125bp; total of 240,578 reads) or that had too many unqualified bases (denoted as “N”; total of 22 reads) were discarded. Those that passed the quality check were merged (R1 and R2) to create one single longer sequence. Upon the first filtering step, the total number of reads per SP was 3.2 million, 3.3 million and 4.0 million. Mothur v1.46.1 (Schloss et al. 2009) was used to assign the reads to the respective sample IDs according to the MID-tag-primers combination. Forward and reverse sequences were checked and a minimum of one mismatch was allowed for the assignment. This step was followed by the demultiplexing of files which produces one fasta file per MID-tag combination (equivalent to each sample). The script also edited the headers by adding the sample ID to the beginning of each sequence. Usearch v11 was used with the Unoise3 command to remove chimeras, denoise the sequences and group identical sequences into zero radius operational taxonomic units (zOTUs). These are generated using a clustering identity threshold of 100% to avoid multiple species being grouped under the same OTU (conservative algorithm). At this stage, read abundance matrices (number of each zOTU reads per sample) were created along with a zOTU list. Taxon information for each taxonomic unit on the list was obtained by comparison with sequences on the most up to date eukaryote nucleotide database from NCBI GenBank (2022). The BLASTn v2.12.0 (Altschul et al. 1990) was used in BLAST+ (Camacho et al. 2009) with a minimum percentage identity score of 96.02%, a value based on the mean value for the average genetic distances of sister species in angiosperms (3.98%, Qin et al. 2017).

From this point onwards, data clean up and further filtering was performed with a combination of functions in excel and manual processing methods. The zOTU list was produced with samples originating from both national parks. However, in some cases, the genus was the same for both study sites but the species was site specific. Hence, criteria to define the taxonomic ID of each zOTU was adjusted to each national park taking into consideration the botanical surveys available for each site. If the plant species was common across both sites or if it only existed in one site, species level was retained. If plant species differed between NPs or if no taxonomic agreement was achieved for each or both sites, genus level was retained. Taxa that were not present in Africa or West Africa were not considered, as well as any fungal or bacterial sequences or other types of contamination (e.g. homo sapiens, starch - *Solanum* spp. originating from the extraction kit spin columns). When minimum requirements for species or genus level identification were not met (not erroneous, high quality or sequences longer than 125bp), taxa were excluded. zOTUs belonging to the same taxa were then aggregated and a final matrix was obtained for the full dataset. Reads originating from contamination, tag jumping and bleeding of over-represented taxa into other samples were removed using the combined maximum read count for blank, extraction negative and MID-tag PCR negative samples (Drake et al. 2022). A per zOTU threshold of 1% was further applied to eliminate low read counts. The value of the threshold was taken from a study that assessed contamination rates in complex field environments where samples are exposed to non-food DNA on the ground (Ando et al. 2018). After these cleaning steps, taxa were reduced by 24% but this more conservative approach was selected so plant diversity detected in the diet of primates would not be inflated.

3.3.4.2 Statistical analysis

Bioinformatic analysis

All statistical analyses were conducted in R v4.1.3 (*R Core Team* 2022). Dietary variation among primate species and sampling sites were visualised with nonmetric multidimensional scaling (NMDS) using the function ‘metaMDS’ in the R package *vegan* (Oksanen et al. 2019). The number of dimensions were extracted from Jaccard distances matrix. Data were plotted with *ggplot2* (Wickham et al. 2016). The read abundance matrix was converted into a presence-absence matrix that included all western red colobus and western chimpanzee samples (N = 430). Information on each sample (e.g. collection site, season, sex) was added to the matrix. This table was used for most statistical analysis, and in some cases, subsets were generated. In this chapter, descriptive statistics were detailed for chimpanzee populations in each NP and compared with previous studies conducted in the same area. A final table indicating the plant taxa detected in the diet of chimpanzees was compiled and the frequency of occurrence (F_o% - number presences across all samples) was calculated for each taxon.

Dietary richness

The Hill-Diversity index, and coverage-based rarefaction and extrapolation were used to quantify dietary diversity and estimate if the number of samples was enough to cover the plant species theoretically present in the chimpanzee’s diet (Hill 1973; Roswell et al. 2021). This method was preferred over sample size and effort-based standardisation, and asymptotic species-accumulation curves because they fail to account for the species-abundance distribution of the community sampled (Roswell et al. 2021; Tercel et al. 2022). Hill diversity is measured as the generalised mean species rarity and three exponents can be used to determine the sensitivity of the equation to rare species. All exponents were tested using the R package *iNEXT*: the most sensitive Hill-richness ($_l = 1$), Hill-Shannon ($_l = 0$) which responds to both high and low rare species, and the least sensitive Hill-richness ($_l = -1$).

Variation in diet composition

To evaluate seasonal variation in dietary composition, a model-based approach in the *mvabund* R package (Wang et al. 2012) using simultaneous GLMs (generalised linear models; function ‘ManyGLM’), a binomial error family and respective link function, and Monte Carlo resampling method were implemented. Data residuals were checked for normality for the modelled GLMs and Likelihood ratio tests (LRT), and resampled *p*-values are presented for the models selected.

3.4 Results

3.4.1 Extraction, amplification and barcoding success

Out of 740 faecal samples collected in the field and extracted in the laboratory, 114 did not amplify with the mitochondrial primers, most likely due to poor DNA quality. Overall amplification success for species identification using mitochondrial markers was 84.6%. The number of samples molecularly identified for each species in each season is presented in Table 3.2.

Table 3.2 Number of samples per species, season, and NP. Samples collected in the field and molecularly identified for each primate species, in each National Park, for each season. Not all of these samples were successfully sequenced with a DNA metabarcoding approach (details in section 3.3.4.1 and 3.4.2). In some cases, sample size is too small to perform statistically robust analysis (e.g. *P. t. verus* in Gola RNP).

	Gola RNP			Cantanhez NP		
Sample	Dry season	Rainy season	Total	Dry season	Rainy season	Total
Piliocolobus badius	98	133	231	88	33	121
Pan troglodytes verus	1	5	6	42	16	58
Other primate species	14	114	128	68	9	77
Unsuccessful amplifications	13	23	36	65	18	83
Total	126	275	401	263	76	339

3.4.2 DNA sequencing and plant identification

A total of 64 *P. t. verus* (Gola RNP, N = 6; Cantanhez NP, N = 58) were amplified with the UniPlant primers. Two *P. t. verus* samples did not amplify and were excluded from the HTS. The UniPlant primer amplification had 96.9% amplification success.

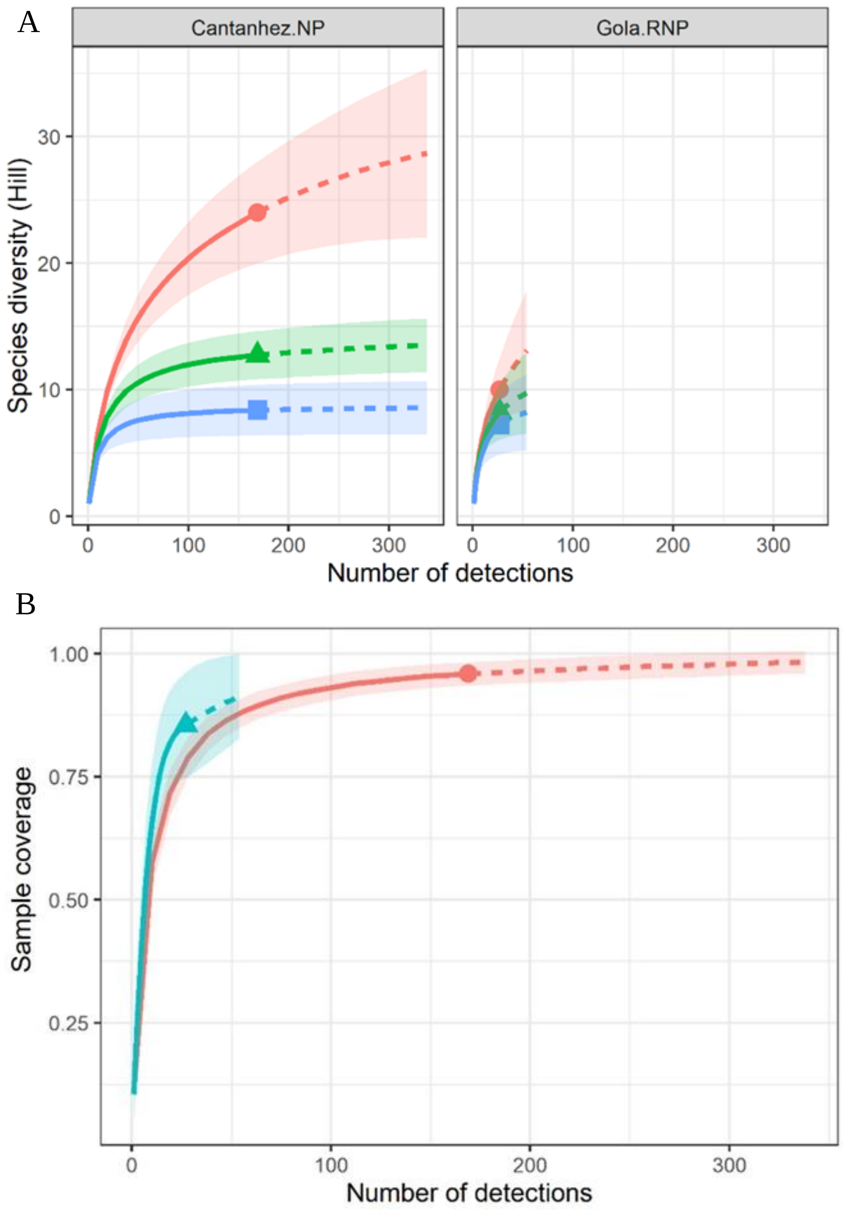
Of the initial 6,967,262 reads obtained across the three SP, 1.7% were identified as reads with sequencing errors or chimeras, and were removed from the dataset. An average of 7,147 DNA reads (±3,593 SE) were obtained per sample, and a total of 14,867 zOTUs were compared with the NCBI GenBank reference database. Several zOTUs were discarded due to no or unsure taxa assignment (9.9%), taxa not native to or introduced in West Africa (1.1%), assigned to bacterial taxa (0.2%) and to fungi (3.4%). This last percentage is relatively high possibly because the marker is also a universal barcode for fungi (Schoch et al. 2012) and some samples had developed fungi during the storage period. During the DNA metabarcoding sample preparation, control negatives, positives and blanks were added to address contamination issues and possible MID-tag jumping during the HTS. We did not detect tag jumping in the MID-tag combinations used in this thesis, but this issue had been previously studied by other researchers in the same lab, that had produced similar DNA metabarcoding projects and indicated which MID-tag combinations should not be used. The maximum contamination detected across the three controls was low (1.0 %), with 4.0 % for negative controls, 0.3 % for blank samples, and 2.2% for positive controls. The combined read maximum count originating from contamination were removed from each DNA sample, producing a dataset with the same number of taxa (zOTU) as the initial dataset (228), but with a much lower count number per zOTU (82.8% reduction in count number per zOTU). Additionally, in order to have a conservative approach to possible contamination, a 1% threshold per zOTU was applied to eliminate low read counts. This step reduced the number of final zOTU detected in the diet of *P. badius and P. t. verus*. Of the 8,753 dietary presence counts found in western red colobus and chimpanzee samples across the three years, 171 plant taxa were identified to species or genus level, belonging to 45 plant families. This thesis produced a dataset with 62 chimpanzee samples with good quality plant reads.

The final dataset for western chimpanzees (*P. t. verus*) comprised six samples collected in Gola RNP, Sierra Leone and 56 in Cantanhez NP, Guinea-Bissau. The number of plant items detected in chimpanzee faecal samples ranged between one and nine, and on average 3.16 (±SE 1.59) plant taxa were identified per sample (details per NP in Table 3.3). A total of 33 plant taxa belonging to 22 families were identified to the species (N = 25) or genus level (N = 8) (Tables 3.4 and 3.5). Of these, 12.1% (N = 4) were agricultural crops: *Abelmoschus* spp., detected in samples from Gola RNP, and mango, cashew and *Citrus* spp. present in samples from Cantanhez NP. Detailed results on the diet of chimpanzees in each national park are presented further below.

Table 3.3 Western chimpanzee plant dietary richness. Number of plant taxa detected in the diet of two populations of *P. t. verus* in West Africa. Plant richness per sample is shown for each NP population, and per season. Total sample size of per NP as in brackets per season (D = dry; R = rainy). Percentage of crop species (% Crops) represents the number of plants detected in chimpanzee diet that is a cultivated item, and in brackets the total frequency of occurrence (TF$_o$%) in faeces. In Gola RNP, crop consumption may be overestimated due to small sample size, but in Cantanhez it is similar to a study conducted in the same NP, which detected a value of 13.6% (Bessa et al. 2015a).

			Per sample					Per study site	
					Plant richness (±SE)			% Crops (TF$_o$%)	
National park	Sample size (D/R)	No. plant taxa	Min.	Max.	Overall	Dry season	Rainy season	Dry season	Rainy season
Gola	6 (1/5)	10	3	7	4.50 (±1.4)	3 (±0.0)	4.8 (±1.8)	0.0	10.0 (80.0)
Cantanhez	56 (40/16)	24	1	9	3.02 (±1.5)	3.2 (±1.5)	2.6 (±1.8)	12.5 (45.0)	7.7 (6.3)

Dietary diversity estimates computed with Hill-richness exponent (l = 1) provided higher diversity estimates than the other two exponents available (Hill-Shannon, l = 0; Hill-Simpson, l = -1) (Fig. 3.5). As Hill-diversity exponent is more sensitive to rare species in our dataset, this result suggests that it was possible to detect a great variety of plants rarely eaten plants by chimpanzees. Sample size, and consequently presence counts, in both NPs differed greatly. However, the estimated coverage of the dietary community of chimpanzees in both sites was high: Gola RNP - 85.5% (±95%CI: 12.8) and Cantanhez NP - 95.9% (±95%CI: 2.6) (Fig. 3.5, panel B).


A
Cantanhez.NP
Gola.RNP
Species diversity (Hill)
0
10
20
30
0
100
200
300
0
100
200
300
Number of detections
B
Sample coverage
0.25
0.50
0.75
1.00
0
100
200
300
Number of detections

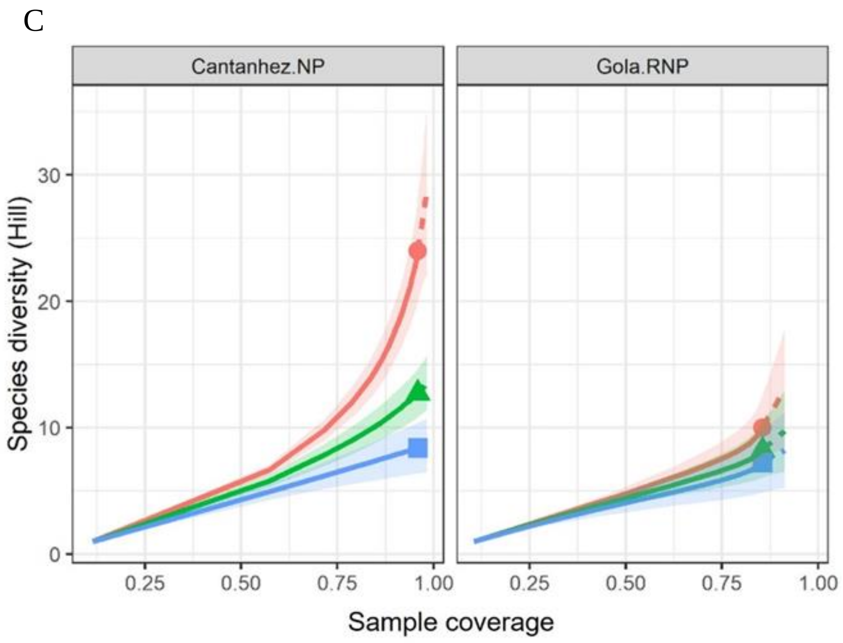


Figure 3.5 Western chimpanzee dietary species diversity and community coverage. The percentage of plant species detected in western chimpanzee non-invasive samples using a DNA metabarcoding approach are estimated with three exponents with different sensitivities to species rarely consumed by *P. t. verus*. Values of the exponent l, which determine the rarity scale of different diversity estimates, are represented in different colours: Hill-richness ($l = 1$), red line with circle; Hill-Shannon ($l = 0$), green line with triangle; Hill-Simpson ($l = -1$), blue line with square. Solid lines are the observed diversity, which is further extrapolated (dashed lines). Shading around the line correspond to 95% confidence intervals. Top image (A) shows the species diversity observed for the total number of dietary detections in chimpanzee populations of Cantanhez NP (left) and Gola RNP (right). Middle graph (B) demonstrates how much of the dietary community was possible to cover in Cantanhez NP (red line with circle) and Gola RNP (blue line with triangle), with the number of detections resultant from the DNA metabarcoding. Bottom image (C) illustrates the species richness per sample coverage in Cantanhez NP (left) and Gola RNP (right).

3.4.3 Dietary composition of *Pan troglodytes verus*

3.4.3.1 in Gola RNP, Sierra Leone

The number of faecal samples collected for chimpanzees in Gola RNP was low, with only one sample collected in the dry season, and thus many dietary comparisons were therefore, not possible to conduct. Nevertheless, 10 plant taxa belonging to eight families were detected (Fig. 3.6) in the six faecal samples. Eight of the ten plants were recorded in previous botanical surveys conducted in the park, but not *Abelmoschus* spp. and *Manilkara obovate*. However, both plants are known to be present in Sierra Leone (POWO 2022). Half of the plant taxa were only detected in one or two samples, but the other half were frequently detected across the dataset (Table 3.4).

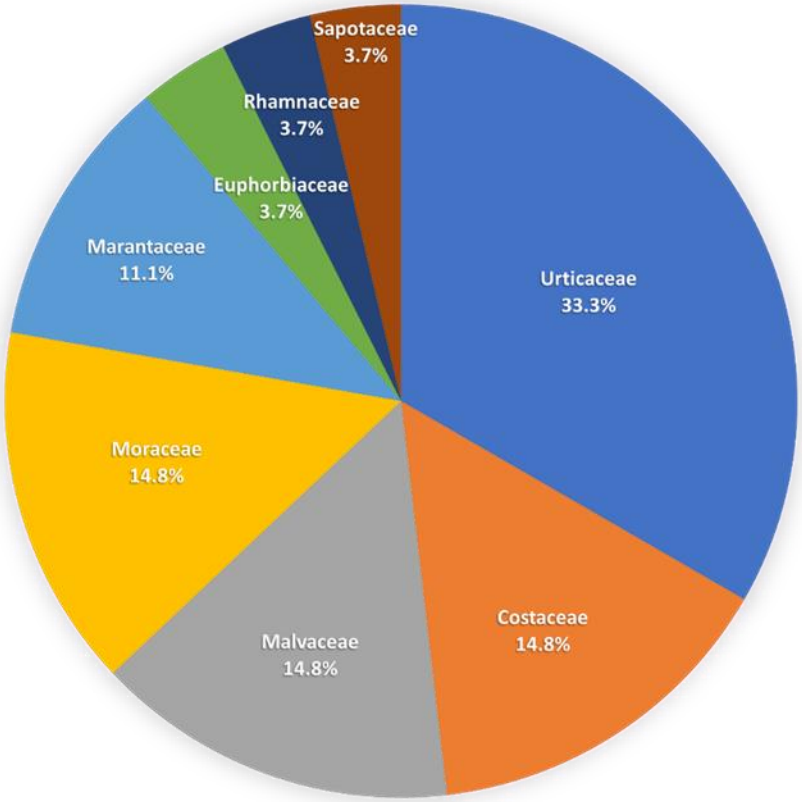


Figure 3.6 Gola RNP chimpanzee dietary composition by plant family. Frequency of occurrence of plants detected in chimpanzee diet per plant family was calculated using the dietary counts. A variety of plant families contribute in similar proportions to the dietary requirements of chimpanzees from Gola RNP. Due to the small sample size, all plant families were comprised in the figure, including those present in only one or two faecal samples.

Fruiting trees and herbs, and one climber, were the most common plants identified in chimpanzee diet in Gola RNP, which was expected considering the species frugivorous diet (see Table A4.1. and A4.2 in Appendix 4). Only one plant species was recorded in all chimpanzee samples, *Musanga cecropioides*. This taxon is known as the umbrella tree and is typically present in secondary forests (Yamakoshi 1998). Among the top five plants was *Abelmoschus* spp., an introduced crop, for which species could not be identified due to low percentage of sequence assignment under the BLASTn bioinformatic step.

Table 3.4 Plant taxa detected in chimpanzee diet at Gola RNP. Characteristics of wild and crop plant taxa detected in chimpanzee non-invasive samples using a DNA metabarcoding approach. * plant taxa found also or exclusively in forest regrowth areas. Life form: climber (C), herb (H), shrub (S), tree (T). Season: dry (D) Dec-Apr; rainy (R) May-Nov. F_o(%): Frequency of occurrence, the number of times plant taxa was detected across samples (N = 6). Part eaten: bark (B), flower (Fl), fruit (Fr), leaves (L), twigs (Tg); this information was obtained from previous studies (references in Table A.3.1 in Appendix 3). Status: IUCN Red List Conservation status, least concerned (LC), not available (NA).

Plant family	Dietary taxon	Life form	Season	F_o (%)	Part eaten	Status
Urticaceae	*Musanga cecropioides**	T	D, R	100.00	L, Fr	LC
Costaceae	*Costus* spp.	H	R	66.67	L, Fl, Tg	NA
Moraceae	*Ficus* spp.	T	D, R	66.67	L, Fr, B	LC
Urticaceae	*Myrianthus arboreus**	T	D, R	50.00	Fr, L	LC
Marantaceae	*Hypselodelphys violacea*	C	R	33.33	Tg	NA
Rhamnaceae	*Gouania longipetala**	S	R	16.67	-	NA
Euphorbiaceae	*Macaranga barteri**	S, T	R	16.67	L, Fr	LC
Sapotaceae	*Manilkara obovata*	T	R	16.67	-	LC
Marantaceae	*Megaphrynium* spp.*	H	R	16.67	Tg	NA
Crops						
Malvaceae	*Abelmoschus* spp.	H	R	66.67	Fr	LC

3.4.3.2 in Cantanhez NP, Guinea-Bissau

DNA metabarcoding detected 24 plant taxa in the faeces of chimpanzees from Cantanhez NP (Guinea-Bissau). These taxa belonged to 18 families which were identified to the species (N = 17) or genus level (N = 7) (Fig. 3.7). Only one of the taxa has not been recorded in the park's botanical surveys, but this food item was strongly assigned to the plant species *Mezoneuron benthamianum* (percentage of identity >96.8%), which is present in Guinea-Bissau (POWO 2022). Similar to Gola RNP, nearly half (41.7%) of the plant taxa in Cantanhez NP chimpanzee faeces was only detected in one or two samples.

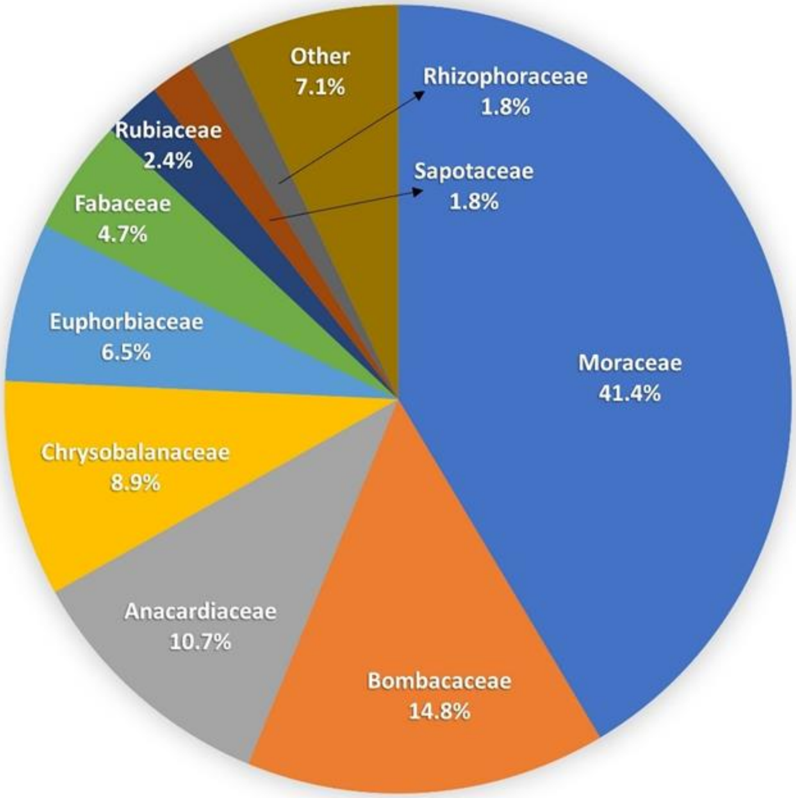


Figure 3.7 Cantanhez NP chimpanzee dietary composition by plant family. Frequency of occurrence of plants detected in chimpanzee diet per plant family was calculated using the dietary counts. Moraceae and Bombaceae make up more than 50% of the chimpanzee diet. For visualisation purposes, nine additional plant families were grouped together under the category 'Other'. These taxa were detected only once or twice across all 56 samples.

The plant taxon with the highest frequency were *Ficus* spp. (78.6%) and the Kapok tree (*Ceiba pentandra*, 44.6%). The two species both contribute fruit, flowers and bark to the chimpanzee diet. Although *Ficus* spp. can be found in a variety of habitats including secondary forests, *Ceiba pentandra* is typically a tree growing in wet evergreen and dry semi-deciduous tropical forests, and in Cantanhez NP this plant is found in nearly all types of habitat. Cultivated mango (*Mangifera indica*) is found among the top five most important plant species for chimpanzees. Overall, 12.5% of the plant taxa detected with DNA metabarcoding were crops (mango F_o = 23.2%, cashew F_o = 8.9%, *Citrus* spp. F_o = 1.8%). Mango and cashew were consumed in the dry season, the time at which trees are fruiting, while *Citrus* spp. was detected in samples collected in the end of the rainy season, which is when trees start producing their fruits. Crop consumption was higher in the dry season, when most wild ripe fruit is available.

Table 3.5 Plant taxa detected in chimpanzee diet at Cantanhez NP. Characteristics for wild or crop plant taxa detected in chimpanzee non-invasive samples using a DNA metabarcoding approach. **Life form**: climber (C), herb (H), shrub (S), tree (T), vine (V). **Season**: dry (D) Dec-May; rainy (R) Jun-Nov. **F_o(%)**: Frequency of occurrence, the number of times plant taxa was detected across samples (N = 56). **Part eaten**: bark (B), flower (Fl), fruit (Fr), leaves(L), pith (Pi), twigs (Tg); this information was obtained from previous studies (references in Table A.3.1 in Appendix 3). **Status**: IUCN Red List Conservation status, data deficient (DD), least concerned (LC), vulnerable (VU), not available (NA).

Plant family	Dietary taxon	Life form	Season	F_o(%)	Part eaten	Status
Moraceae	*Ficus* spp.	T	D, R	78.57	L, Fr, B	LC
Bombacaceae	*Ceiba pentandra*	T	D, R	44.64	Fr, Fl, B	LC
Moraceae	*Milicia regia*	T	D	23.21	Fr, L	VU
Moraceae	*Treculia africana*	T	D	23.21	Fr	LC
Euphorbiaceae	*Phyllanthus muellerianus*	S, V	D	19.64	Fr	NA

Chrysobalanaceae	*Neocarya macrophylla*	T	D, R	16.07	Fr	NA
Chrysobalanaceae	*Parinari excelsa*	T	D, R	10.71	L	LC
Rubiaceae	*Mussaenda elegans*	S	R	7.14	-	NA
Fabaceae	*Vigna* spp.	H	D, R	7.14	L	LC
Rhizophoraceae	*Anisophyllea laurina*	T	D	5.36	Fr	LC
Fabaceae	*Dalbergia* spp.	T, S, C	D	5.36	L	LC
Sapotaceae	*Mimusops adongensis*	T	R	5.36	Fr	LC
Cannabaceae	*Trema orientalis*	S	R	3.57	Fr	LC
Malvaceae	*Triumfetta* spp.	S, H	R	3.57	L	NA
Urticaceae	*Urera oblongifolia*	V	R	3.57	L, Pi	NA
Vitaceae	*Ampelocissus* spp.	V	R	1.79	Fr	NA
Convolvulaceae	*Merremia* spp.	H, S	R	1.79	L	NA
Fabaceae	*Mezoneuron benthamianum*	V	D	1.79	L	NA
Simaroubaceae	*Odyendea klaineana*	T	D	1.79	-	NA
Dilleniaceae	*Tetracera potatoria*	T	D	1.79	L	NA
Meliaceae	*Trichilia prieureana*	T	D	1.79	Fr	LC
Crops						
Anacardiaceae	*Mangifera indica*	T	D	23.21	Fr	DD
Anacardiaceae	*Anacardium occidentale*	T	D	8.93	Fr	LC
Rutaceae	*Citrus* spp.	T	R	1.79	Fr	LC

The simultaneous MGLMs modelled using the mvabund R package indicated a significant difference in the diet composition of chimpanzee samples collected in the dry and in the rainy season in Cantanhez NP (LRT = 103.8, $p = 0.001$). This difference can be visualized in the NMDS plot (stress value = 0.15) (Fig. 3.8). Univariate tests identified five plants with significant GLM results, four of which detected in the dry season only, when fruit availability in the park is at its lowest. One of the plant species is the crop mango, highly detected in chimpanzee faeces (Table 3.6).

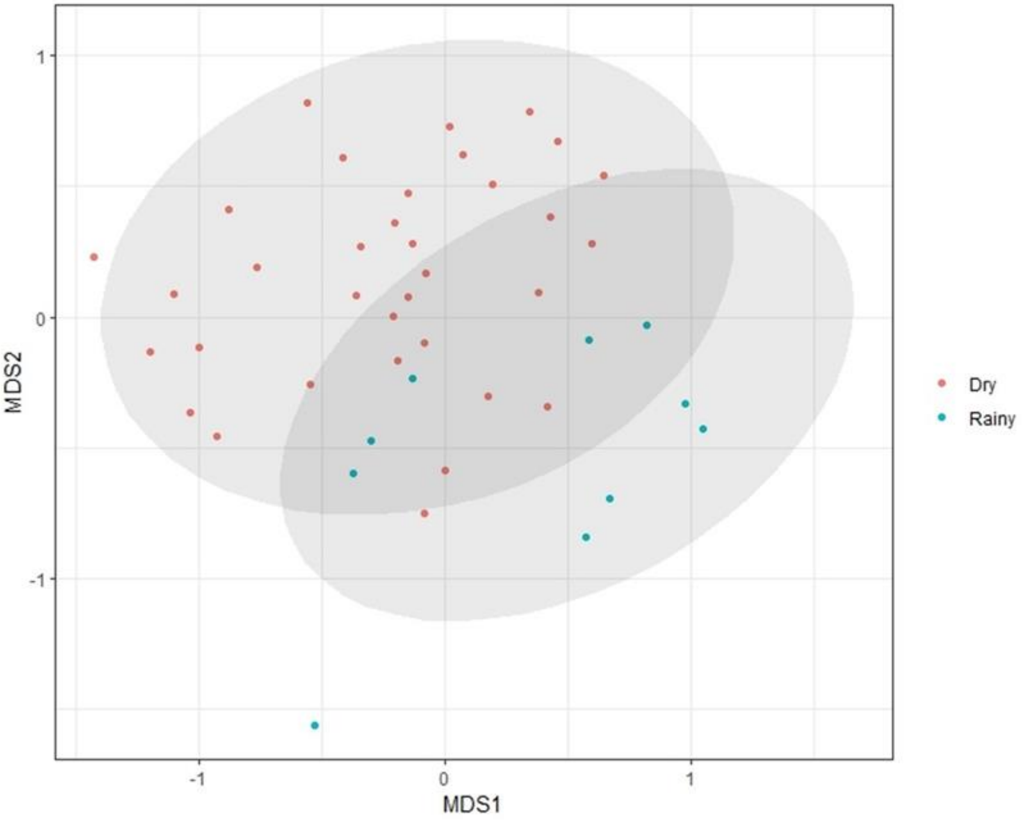


Figure 3.8 Chimpanzee dietary variation across seasons in Cantanhez NP. Pairwise biplots from non-metric multidimensional scaling (NMDS) analysis represent a significant variation in dietary composition of chimpanzees between the dry season (red dots, elipse on the left) and the rainy season (blue dots, elipse on the right) in Cantanhez NP.

Table 3.6 Analysis of variance in chimpanzee diet across seasons in Cantanhez NP. Univariate tests results for plant taxa which contributed significantly for the variation in diet observed between the dry and rainy seasons.

Plant taxon	Fruiting	Season detected	LRT	*p*-value
Mangifera indica ©	Feb-Aug	D	10.24	0.023
Milicia regia	Apr-Jun	D	10.24	0.022
Mussaenda elegans	NA	R	10.83	0.017
Phyllanthus muellerianus	Feb-Aug	D	8.43	0.038
Treculia africana	Dec-May+Jul	D	10.24	0.023

LRT: Likelihood ratio test value and respective p-value. ©: crop species.

3.5 Discussion

In general, DNA metabarcoding sequencing of the ITS2 plant primer proved successful in non-invasive samples of a critically endangered ape. zOTU read counts above zero were obtained for 62 western chimpanzee samples, indicating 96.9% UniPlant amplification and HTS success. The number of plant taxa identified in the faeces (N = 33) was not the highest recorded when compared with studies conducted at other sites (minimum number recorded was 32 with maximum 200). However, in contrast to our study (Gola RNP: N = 6 faeces across 5 months; Cantanhez NP: N = 56 faeces across 3 months), previous research used observational, faecal matter examination and/or feeding traces detected, and not molecular techniques, for extensive periods of time and across many faecal samples (Table A.3.1 in Appendix 3). This observation is discussed further below upon a closer comparison with one of the chimpanzee communities of Cantanhez NP.

The estimated diversity at both study sites, albeit only six samples in Gola RNP, suggest that chimpanzees do not consume plant species randomly. This finding corroborates the description of chimpanzee as ripe fruit specialists (Wrangham 1977). Yet, the number of samples was not enough to completely cover the estimated dietary community, leaving a gap of 15.5% and 4.1% of undetected plant species in the diet of chimpanzees in Gola RNP and Cantanhez NP, respectively. Nevertheless, our results suggest that using a DNA metabarcoding approach covers a high percentage of the dietary community of chimpanzees, including species rarely eaten (Hill-richness $_l$ = 1).

3.5.1 Chimpanzee diet in Gola RNP

The chimpanzee population in Gola RNP has a low density and as a result, a low number of samples was collected in the field and identified as *P. t. verus*. Still, a higher percentage of the theoretical dietary diversity of the population was covered in this study. No other dietary studies have been developed in this population of chimpanzees nor in Sierra Leone, but comparisons can be made with other research conducted on the same subspecies at other sites of West Africa. All but one of the plant taxa detected in faeces were previously identified as food that chimpanzees consume at other study sites (Sugiyama and Koman 1992; Yamakoshi 1998; Hockings and McLennan 2012). The one that records of being consumed were not found in literature, *Manilkara obovata*, produces fruits that could be serve as food to chimpanzees. However, the detection of this species could be a result of accidental consumption, as its twigs are used as ant-feeding tools (Koops et al. 2015). Half of the plants identified in the diet of chimpanzee from Gola RNP, grow in secondary forest. In fact, *Musanga cecropioides* which is typically present in secondary forests, with some exceptions, was present in all six chimpanzee faeces. The result appears to suggest that chimpanzees forage more in forest regrowth and depend highly on plants characteristic of these type of habitat. However, sample location must be considered. Four of the six samples were collected in the community forest which is an area of secondary forest, and the other two faeces could have been collected in old logging areas where forest regrowth takes place. One further explanation for the high presence of *M. cecropioides* in chimpanzee diet, could result from the availability of fruits through most of the year. This food item has been recorded to be consumed by chimpanzees as a response to fruit scarcity (Yamakoshi 1998; Takemoto 2003), and it could also be the case in Gola RNP.

One cultivated food was frequently identified in chimpanzee faeces. *Abelmoschus* spp. has been previously identified in the diet of chimpanzees from Bossou, Guinea (Hockings and McLennan 2012). This zOTU resulted in four top hit species of the *Abelmoschus* genera, with percentage identities between 97.7% and 100%. However, the likelihood of being the okra species *A. esculentus* (average identity of 99.7%) is high, given that this plant is a cultigen (plant altered by humans through selective breeding) grown in Africa for centuries and is widely cultivated for its edible fruits (POWO 2022), including Gola region (pers. obs.). The reason for the high percentage of this crop (66.7%) detected in chimpanzee diet can be explained by the fact that all samples containing okra were collected in the same location indicating the same group of chimpanzees, which could have been feeding in the community forest, where humans have cultivated fields.

Due to the low number of samples collected in Gola RNP, it was not possible to perform a dietary composition seasonal analysis. However, the three plant taxa detected in the only sample collected in the dry season were also found in samples collected in the rainy season. These were identified as trees that produce fruit in both dry and rainy season. Most of the remaining plant taxa identified in samples collected in the rainy season diet are consumed in the form of leaves, flowers, bark and twigs. This is the season of less fruit availability in Gola RNP. The result supports the observation that chimpanzees diversify their diet in times of fruit shortage (Wrangham 1977; Doran-Sheehy et al. 2006; McLennan 2013).

3.5.2 The diet of Cantanhez NP chimpanzees

Plant richness in the diet of chimpanzees from Cantanhez NP is within the expected values of what has been described in other studies for the different subspecies. The number of plants detected in this study was very similar to the study on *P. t. verus* of Lagoas de Cufada Natural Park (33 wild species plus 9 crops, Carvalho et al. 2015), and on *P. t. schweinfurthii* group in Montane forest in Bwindi, Uganda that found 32 plant species (Stanford and Nkurunungi 2003). However, for the first study 210 faecal samples collected only in one dry season were analysed, and in Uganda, field work was conducted over a four times longer period than the current study, and the number of faecal samples collected was considerably larger (N = 187). Similarly, the only dietary study carried out in one of the 12 chimpanzee communities of Cantanhez NP (Caiquene-Cadique chimpanzee community) was also conducted for 9 months and involved faecal examination of 377 samples (Bessa et al. 2015b). This research identified 66 plant species in chimpanzee faeces, belonging to 28 plant families. Even though the current study covered a wider area of the national park aiming at sampling more communities of the Cantanhez chimpanzee population, sample size was much lower (N = 56). The current study, which involved collecting faecal samples over three months, identified 24 plant taxa in chimpanzee diet across the park. The raw number of plants in comparison with Bessa et al. (2015) research is lower, but this is likely explained by the lower sampling size and smaller number of months. Nevertheless, frequencies of occurrence in this study were generally higher than the ones obtained in the 2015 study (e.g. *Ficus* spp.: 2015 = 45.1%, 2019 = 78.6%; *Ceiba pentandra*: 2015 = 10.3%, 2019 = 44.6%; *Treculia africana*: 2015 = 11.4%, 2019 = 23.2%). Overall, the result suggests that the DNA-based method can provide a good characterisation of chimpanzee diet and if more samples are collected for a longer period, a better coverage of the species consumed by *P. t. verus* will be achieved.

As proposed in our second prediction, and in accordance with other studies on chimpanzee diet (Wrangham 1977; Morgan and Sanz 2006; Chancellor et al. 2012; McLennan 2013), seasonal variation was strong in the Cantanhez population. We assume this result is believed to be direct consequence of the sampling period. Fruit availability surveys conducted by (Bessa et al. 2015b) registered a fruiting peak in March and the lowest availability in November, and these are the exact periods when sampling for this study took place. Variation could be explained by seven plants, which all had seasonal fruiting. *Milicia regia* and *Treculia africana*, together with mango, all produce fruits in the dry season, the period when fruit availability is highest in the park. Moreover, the three plants were frequently detected in the diet (23.1%) suggesting that these plants are important and preferred chimpanzee foods. However, the mean number of plant taxa detected in the rainy season was lower than in the dry season. This is an unexpected result as chimpanzees have been reported to increase dietary diversity during periods of fruit scarcity (Fawcett 2000; McLennan 2010).

The most important plant species for Cantanhez chimpanzees consist of fruiting trees and shrubs. The two plants with the highest frequency of occurrence were *Ficus* spp. (78.6%) and *Ceiba pentandra* (44.6%), that fruit most of the year and for which chimpanzees eat both fruits and leaves (Bessa et al. 2015b; McLennan et al. 2020). These plant taxa are found in evergreen and dry-deciduous rainforests, but in Cantanhez they have been recorded across most habitat types. The higher frequency of these plants in the primates diet reflects the capacity of chimpanzees to forage in different habitats and adapt their dietary requirements to the surrounding environment. Half of the plant taxa detected in this study had been previously identified as food for the Caiquene-Cadique chimpanzee community and the population of Lagoas de Cufada Natural Park, also in Guinea-Bissau and a human-modified landscape (Carvalho et al. 2015). The remaining plant taxa have been recorded as food for other chimpanzee communities across Africa (Table 3.5). Some were fruits (Wrangham 1977; Sugiyama and Koman 1992), and others were leaves and pith (Takemoto 2003; Carvalho et al. 2015; McLennan et al. 2020), with a higher number of plants being consumed by Cantanhez chimpanzees in the dry season. These plants might be consumed as a response to low fruit availability or simply as a preference for specific or rare species (Rogers et al. 2004). In two cases, we could not find evidence in the literature that these plants are eaten by chimpanzees. The shrub *Mussaenda elegans* generates scarlet or orange flowers, and the *Odyendea klaineana* tree has black ripe fruits and yellowish-cream flowers that are sweetly scented. If chimpanzees fed only on these species' flowers, the plants could have been missed upon examination of plant parts in faeces.

Three crop plants were detected using a DNA metabarcoding approach: mango (*Mangifera indica*) and cashew (*Anacardium occidentalis*) which fruit in the dry season, and *Citrus* spp. fruiting in the rainy season. These are commonly consumed by chimpanzees across other anthropogenic landscapes (Hockings and McLennan 2012) and the rate of crop consumption revealed in this study (12.5%) is close to the one observed by Bessa et al (2015) (13.6%) and in other populations (Bossou – 12%, Hockings et al. 2009; Bulindi -14%, McLennan 2010). However, the frequency of detections of these items in faeces was much higher in this study than in the 2015 one (33.9% and 3.98%, respectively). This shows once more, the efficiency of DNA metabarcoding method. Additionally, our results appear to corroborate the idea that in Cantanhez NP crops are a preferred chimpanzee food rather than an alternative because of scarcity of wild fruit. This is because the most consumed cultivated species detected in this study were consumed when wild fruit availability was at its highest in the park (dry season). Lastly, our expectation (prediction no. 3) that chimpanzees in Cantanhez NP would consume a larger number of crops due to higher exposure to cultivated land, was supported by our data, although this was not a large difference (crop consumption in Gola RNP = 11%; in Cantanhez NP = 12.5%). It is important, nevertheless, to treat these results with caution as sample size in Gola RNP is low.

3.5.3 Methodological limitations

DNA metabarcoding is a technique that has been extensively reviewed and its limitations have been described by many authors (Nielsen et al. 2018; Taberlet et al. 2018; Lamb et al. 2019; Arstingstall et al. 2021). Quantification of biomass consumed using this method is a contentious issue and not well accepted by all authors. This is particularly true for plant material as the digestion bias of the different plant species and parts consumed can largely vary, and to date, sequencing output correlates very weakly with food biomass (Deagle et al. 2019; Lamb et al.

2019; Piñol et al. 2019). In this case, the challenge of quantifying the plant taxa that are consumed by chimpanzees is a constraint that can be partially overcome by calculating the frequency of occurrence of each taxon in faecal samples. Thus, frequency of occurrence indicates if the food item is consumed across the population but it does not determine how much it contributes to the nutrition of the consumer.

ITS2 is an efficient primer to sequence plant DNA and the reference database is improving with new research being developed (Chen et al. 2010), yet, the marker is also a universal barcode for fungi (Schoch et al. 2012) and we could confirm this from the large percentage of fungal sequences detected. This suggests an improvement that could be achieved during the preservation stage of samples. Complete desiccation of some samples in this project was not achieved when using only silica in the storage process. Hence, fungal growth was observed and it may have had an effect on the final HTS results. Because chimpanzee samples usually contain considerable moisture, it may be better to use the “two-step” method, in which samples are soaked in >96% ethanol for 24-48 hours and posterior desiccation in silica beads is facilitated (Roeder et al. 2004) rather than simply using silica to dry the samples. Besides improving DNA yield, water content is more efficiently removed than simply storing samples in silica, avoiding fungal growth especially when samples are stored for long periods in field conditions.

Feeding ecology is a field of research that benefits from an integrative approach in the field and in the laboratory, as all methods used to study the diet of species fail to provide complete information on the food items consumed due to technical or funding limitations (Hohmann et al. 2012). Even though DNA metabarcoding allows the user to carry out research in wider regions and obtain more information from a smaller number of samples, without phenological, botanical and behavioural studies many conclusions are difficult to draw. This molecular technique only captures the food items consumed in recent hours or days, depending on the digestion time of the species. Hence, a wider period of faecal samples collection would improve the dietary diversity results of the project. Furthermore, assigning taxonomic information to zOTUs was in some cases ambiguous and resulted in unidentified taxa or not identified to the species level (e.g. *Ficus* spp.). Creating specific plant taxonomic databases for the geographic region of the study would narrow the possibilities and improve results obtained at BLASTn step (Elbrecht et al. 2016; Fahner et al. 2016; Schenekar et al. 2020).

3.5.4 Advantages for Conservation

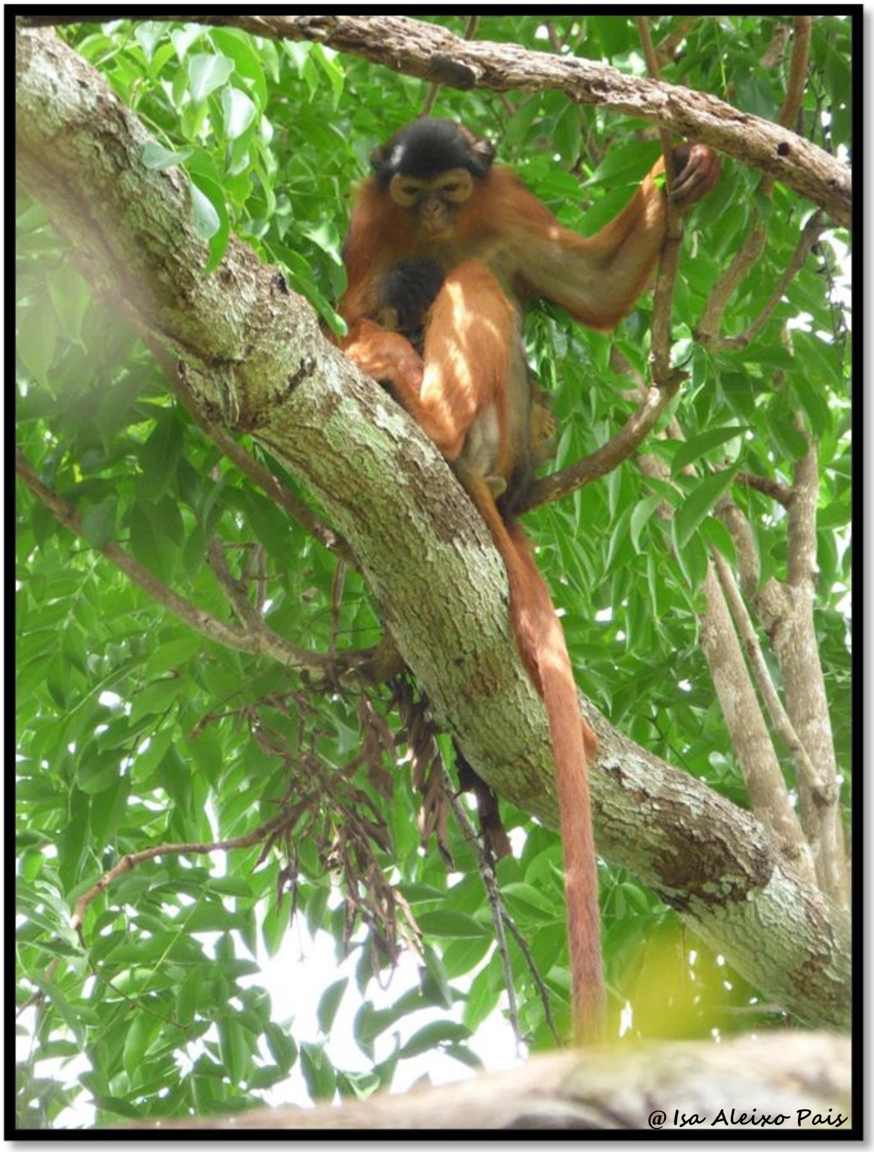
Research is imperative to assist primate conservation, and investigation in the field of feeding ecology will be relevant to understand the evolution of primates and their interaction with the environment, particularly in anthropogenic landscapes. Traditional methodologies commonly applied in dietary studies contribute with important information on quantity and quality (plant parts and species) of food items consumed by these threatened species. However, most populations are unhabituated and research requiring proximity is not feasible. Morphological examination of non-invasive samples is a good alternative, but it loses information due to the degradation of items during the digestion process. DNA metabarcoding provides a faster and more affordable alternative to carry out preliminary research, usually broadens the dietary diversity estimates of previously studied populations, and identifies food items present in low abundance or rarely eaten by individuals.

The method presented here could provide a beneficial approach for the integration of the work performed within the NPs and research centres with molecular laboratories. Sample collection could be carried out opportunistically by the NPs teams and DNA metabarcoding performed at a later stage. This would enable studies to collect larger datasets, cover wider geographic regions and spread research over longer periods. Obtaining detailed data on preferred or most important dietary plant species could be used as a tool for forest conservation and reforestation programmes.

For the chimpanzee populations at Gola RNP and Cantanhez NP, it was important to identify the most important plants in their diet, particularly in the most disturbed landscape. The plants contributing most to the diet of chimpanzees could be thought of as a candidate plants to be included in reforestation and forest regenerating programs. *Musanga cecropioides* and *Ficus* spp, are species that can grow in secondary forests (Catarino et al. 2006; *PROTA4U* 2022), which would help increase the forest cover or prevent forest lost in these areas, and benefit the primates populations as they may serve as fallback or staple foods (McLennan 2010; Bessa 2014). Furthermore, products of the two trees are used by people for food, traditional medicine and construction. Hence, campaigns on the most sustainable extractive procedures and times of the year when harvest would be less impactful to primates should be conducted.

The detection of crops in chimpanzee diet at both sites, and the confirmation that in Cantanhez NP some cultivated items may be preferred rather than fallback foods, indicates a potential for human-wildlife conflict. Further quantitative research on crop damage, and dietary or economic importance of the cultivars to humans would be crucial to design mitigation strategies to reduce the negative impact that primates might have on crops and that encounters with humans may have on primates (Hill 2000; Hockings and Humle 2009; Wallace and Hill 2012). By working with local farmers, camera traps could be used to understand which could be the best deterrent to crop feeding on human livelihoods (Zak and Riley 2017).

CHAPTER 4. Ecological and temporal variation in the diet of the two western red colobus (*Piliocolobus badius*) subspecies



Temminck's red colobus (*Piliocolobus badius temminckii*)
in Cantanhez National Park

4.1 Abstract

The dietary diversity of the western red colobus (*Piliocolobus badius*) living in the continuous forest of Gola Rainforest National Park and in the fragmented habitat of Cantanhez National Park was characterised and differences evaluated between locations, seasons, sexes and time points. DNA metabarcoding was used to obtain high resolution dietary diversity data and multivariate generalised linear models facilitated the analysis of variation in the diet of *P. b. badius* in Gola and *P. b. temminckii* in Cantanhez. The populations at both parks fed on a high number of different plant species and the type of plants consumed varied significantly between the dry and rainy seasons. The diet of females and males in Gola forest (Sierra Leone) did not differ significantly, but significant temporal dietary variance was detected in the forests of Cantanhez (Guinea-Bissau). Higher species richness was observed in the continuous forest of Gola, with four plant taxa contributing to the bulk of the diet of *P. b. badius*. In the fragmented forest of Cantanhez, one plant species (*Treculia africana*) was by far the dominant plant consumed. This tree is not among the most common plants in Cantanhez and it is only present in dense and riparian forests, supporting the idea that *P. b. temminckii* live in isolated groups in the small forest pockets of the park. This population also consumed cultivated food (11% of diet), such as mango, possibly because their nutritional requirements are not fulfilled by the wild species available in the forest fragments alone. *P. b. badius* ingested 3.2% of cultivated food items (e.g. *Coffea* spp.), but the four wild plants contributing the most to this species diet are taxa predominantly present in moist evergreen rainforest, such as *Lophira alata* and *Maranthes aubrevillei*. This is the first dietary study for red colobus of the two national parks and the data produced will be valuable for the implementation of future conservation actions, such as reforestation programmes or species-specific protection. It is clear that an integrative approach including phenology and behavioural research is essential for the successful conservation of western red colobus subspecies.

4.2 Introduction

4.2.1 Background to study

Colobines have evolved and adapted anatomically and physiologically to feed on low-quality food (Davies and Oates 1994). This consists of tough, fibrous and heavily chemically defended food (naturally defended by antifeedants), which is fermented and digested for long periods in the complex quadripartite sacculated stomachs of colobus monkeys (four ‘chambers’), with the help of the gut microbiome (Fleagle 2013). Leaves, which are common and relatively evenly distributed in forests, and seeds, make up the bulk of colobus monkeys’ diet (Maisels et al. 1994). Theoretically, a combination of broad environmental and biological characteristics could permit colobines to explore a wider range of food and habitat than other primates. Nevertheless, the diet of these primates shows some degree of specialisation, varying annually, seasonally and within and among groups (Struhsaker 2010). Extensive research on feeding ecology of red colobus has been conducted on Eastern African taxa, but far less has been developed for those of West Africa.

4.2.2 The primate-environment relationship

The feeding ecology of primates represents a dynamic relationship between the primate itself and its environment, and the resultant responses which are constrained by morphology and physiology (Figure 4.1, Robbins and Hohmann 2006). Food distribution and abundance is understood to strongly influence primate density, population dynamics, activity budgets, and relationships between individuals (Chapman et al. 2004; van Schaik and Brockman 2005; Marshall and Leighton 2006). The ecological-constraints model suggests that within-group feeding competition increases with increased group size, leading to a wider daily travel range in search of food, and in its turn, constraining group size (Milton 1984). Behavioural and ecological studies have shown that spider monkeys divide and recombine into different subgroups in response to availability of dispersed fruits; hamadryas baboons travel long distances daily to obtain food in their harsh environment, whereas gorillas simply require moving a few hundred metres. In contrast, aggression rates in mangabeys rise when feeding on large jackfruits in trees aggregated in specific forest locations (reviewed in Harding 1981). When studying patterns of female aggression in several primate species, Isbell (1991) suggested that in times of food shortage, female aggression was mainly directed at females from other groups except when food was clumped. Then, within group competition would increase. To regulate feeding competition and provide resource exploitation flexibility, for example, chimpanzees adopted a fission-fusion social organisation, where their main group subdivides into smaller communities ("parties") over short time periods (Chapman 1994; Lehmann and Boesch 2004). Resource availability can also influence habitat utilisation and activity patterns, such as time spent feeding, resting, travelling and in social behaviour. For example, herbaceous plants are a low-quality plant type heavily present in the diet of a Zanzibar red colobus group (*Piliocolobus kirkii*). This plant was particularly consumed when tree foods were scarce, and individuals spent less time moving, and more time grooming and in aggression activities, than the forest group where tree foods were more available (Siex 2003).

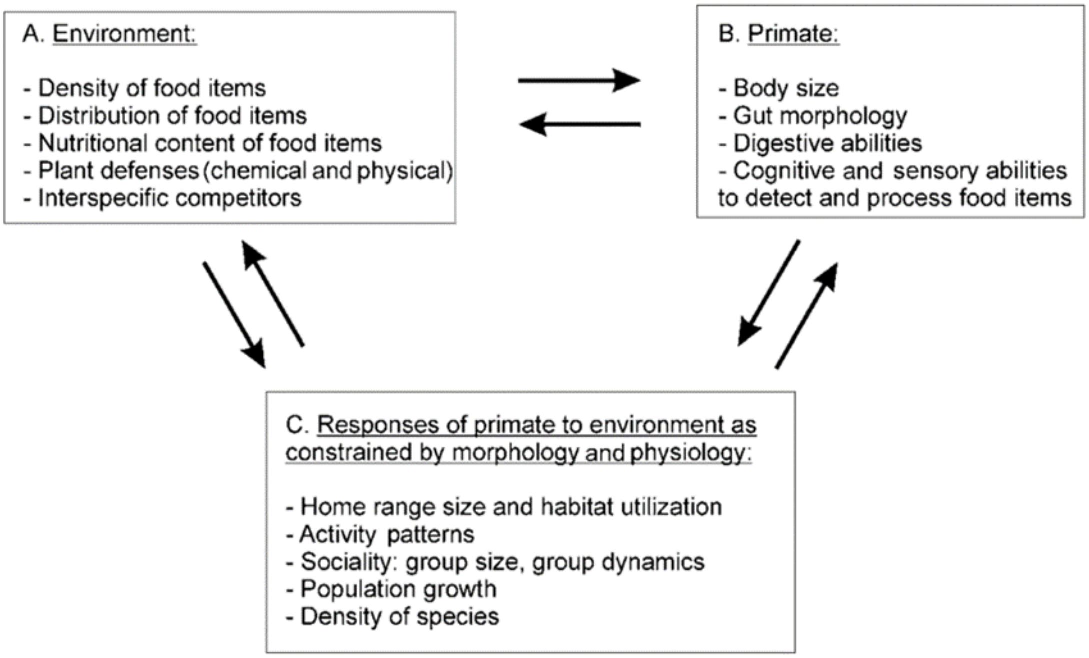


Figure 4.1 A schematic representation of the socioecological model where the relationships between the primate, its environment and the possible responses of a primate to its environment are described (figure from Robbins and Hohmann 2006).

Although the environment may determine the food items in primate diet, depending on its capacity of finding and processing food resources, primates are also capable of influencing their surrounding environment. Many are seed dispersers and capable of influencing plant demography, genetics, spatial distribution and ultimately vegetation composition of habitats (Chapman and Dunham 2018). Besides dispersing an incredibly high number of seeds (e.g. gibbons disperse a minimum of 16,400 seeds per km^2 each year from 160 plant species (McConkey and O’Farrill 2016)), strictly frugivorous monkeys increase plant germination by 75% upon gut passage (Fuzessy et al. 2016). Primates can also control invasive species (e.g. plants and insects), as well as induce the evolution of plant defences against consumption (Robbins and Hohmann 2006). More recently, a study concluded that a change in the spatial distribution of nutrients on the forest floor resultant from red colobus faecal matter contributed to the growth success of light-demanding plant species in Kibale, Uganda (Kalbitzer et al. 2019).

4.2.3 Dietary variability in colobines

The bidirectional relationship between the environment and primates, may result in spatial and temporal variability. Diet can be difficult to determine as dietary variation occurs daily, seasonally, and annually, depending on shifts in the habitat vegetation and flowering / fruiting periods. Inter and intra-species variation in dietary profiles of primates is commonly observed, even in neighbouring groups of the same species that use different home ranges. In a long term study carried out in two groups of ashy red colobus (*Piliocolobus tephrosceles*) in Kibale, Uganda, Struhsaker (2010) identified clear dietary differences, even though the groups were living in the same continuous forest within ~10 km of each other. Apart from distinct differences in tree-species habitat composition, individuals of each group showed different preferences for food species and plant parts.

Patterns of food selection and avoidance in primates have countless causal factors, but it can only be a successful strategy if the nutritional requirements of the forager can be fulfilled with sufficient energy and other nutrients from the items of choice (Oftedal 1991). To understand better which factors influence the variation in primate diet, more information is required on several aspects, such as morphology and behaviour of primates, habitat structure and phenology, and, equally important, food item nutritional and mechanical properties (McGraw and Daegling 2012). The integrative work by Rodman (2002) stresses the importance of good plant taxonomy and the value of plant diversity (food lists) for comparing the diet of primates. His research implies that differences in food selection between African and Asian apes reflect patterns of forest composition and not individual or group preferences for certain plant food species. The recent molecular method of DNA metabarcoding presented in chapter 3, can be a great tool to improve and expand the food item lists required to better understand the diet of primates, particularly those which are threatened and live in heavily disturbed and anthropogenic habitats.

Colobines differ from all other cercopithecines, and primates generally, because they have a large, multi-chambered stomach (Davies and Oates 1994). This unique digestive system combined with enlarged salivary glands and high-cusped molar teeth enables colobines to explore and subsist on difficult to digest, low-quality food items (Oates and Nash 2011). Early studies identified colobines as leaf-eating monkeys (Davies and Oates 1994), requiring the protein and fibre content of foods. However, further field research made clear that colobus monkeys, including red colobus (*Piliocolobus*), feed mainly on leaf buds, flowers or immature seeds and fruits, turning to mature leaves in times of preferred food shortage (Maisels et al. 1994; Oates 1994; Koenig and Borries 2001).

Red colobus often feed in the largest and more common tree species in their habitat, which are frequently spatially and temporally clumped but widely dispersed. This possibly explains the high population densities and extensive home range of some taxa (e.g. *P. tephrosceles* home range = 49 ha; *P. b. temminckii* home range is 34 ha for a group of 24-30 individuals - Starin 1991; Oates 1994), except when constrained by habitat fragmentation. Densities of three red colobus species, *P. kirkii*, *P. rufomitratus* and *P. tephrosceles* from East Africa, are positively correlated with the presence of preferred tree species (Chapman and Chapman 1999; Siex and Struhsaker 1999; Mbora and Meikle 2004). *Piliocolobus* diet is highly diverse not only in plant parts but also species. Diversity is maintained throughout the year and appears not to correlate significantly with ripe fruit availability (Conklin-Brittain et al. 1998; Wrangham et al. 1998; Chapman et al. 1999). However, a small number of resource species contribute to the bulk of red colobus diet, a trait of specialist species (Chapman and Chapman 2002). Red colobus populations of Uganda and Tanzania rely on two or three major food resource, namely *Celtis durandii,* a deciduous, tall tree that provides young leaves, flowers, floral buds, and fruits with seeds (Clutton-Brock 1974; Chapman and Chapman 2002; Struhsaker 2010; Ryan et al. 2013).

Seasonal and annual variation in food availability, implies variation in the diet of red colobus at the species and group levels (Struhsaker 2010). Year-round availability of digestible mature leaves when other more preferred items are scarce (ecological-constraints model) has been proposed to limit group size in folivorous monkeys (Milton 1984; Chapman 2000), with smaller groups being more advantageous when the costs of finding food are not repaid (Chapman 2000; Chapman and Chapman 2000; Chapman and Pavelka 2005). Feeding competition has not been closely studied in African colobines, but it is more likely to occur within the same species or with other colobines, and in larger groups of individuals, as other potential competitors are smaller in size, have lower densities or dietary overlap is low (Oates 1994; Gillespie and Chapman 2001).

Piliocolobus, like other primates, supplement their diet with nutritious and easily digestible food items when ecological conditions occur naturally or are caused by human actions (Mowry et al. 1996; Chapman and Chapman 2002). In some cases, such foods are simply other plant parts or arthropods, but in more degraded habitats, cultivated species are also consumed (Galat-Luong and Galat 2005; van Schaik and Brockman 2005). The well-known studied population of ashy red colobus living in a forest-farm mosaic region in Tanzania, fed mostly on wild foods (98.4%) and more frequently in forests than in farms, but included cultivated bean seeds in their diet changing their behaviour to visit the farms when human presence was low (early mornings and late evenings) (Kibaja 2014).

4.2.4 Conservation relevance

Red colobus are flexible and may adapt their behaviour and diet to changes in the environment, but the question of how long can they survive without access to additional habitat and other vegetation remains (Nowak and Lee 2013). Some primates can feed on fewer types of foods but increase the quantity of those more commonly available in the habitat or increase the dietary diversity in case their preferred items are less abundant. Black-and-white ruffed lemurs included more shrubs and invasive trees in their diet after a cyclone destroyed >50% of their preferred food (Ratsimbazafy 2002). However, they also lost body mass, delayed reproduction, reduced travel distance and spend more time resting (cited in Irwin et al. 2010). So, dietary diversity can act as a buffer against extinction but does not guarantee survival of the species, and it should be treated as a facultative behavioural change (Nowak and Lee 2013).

The importance of gathering information on primate feeding ecology is an important facet for their conservation. As concluded from several dietary studies, it is valuable to obtain information on the type of plants that contribute the most to the diet of specialist primates, such as red colobus. Such studies should focus on primate species and on specific populations that are exposed to alterations to their habitat. Colobines are morphologically adapted to feed on low-quality food throughout the year, and theoretically, would be expected to explore, and possibly survive, in a wider range of habitats. Shifts in the types and quantities of food have been observed in some red colobus populations, demonstrating a certain degree of dietary flexibility. For example, *Pililocolobus badius temminckii* in Fathala forest in Senegal, started feeding on grasses, grass seeds, herbaceous plants and groundnuts in response to a reduction of forest cover (Galat-Luong and Galat 2005). *P. tephrosceles* living in a forest-farm mosaic in Tanzania, fed mostly on wild plants but included cultivated beans probably as a response to a drastic reduction in the abundance of food trees (Kibaja 2014). However, as their diet consists of less nutritious food and more difficult to digest items, red colobus need to ingest larger amounts of food to guarantee minimum dietary requirements (Struhsaker 2010). Hence, this taxon must have access to large quantities of seeds, leaves and unripe fruit. Moreover, their dependence on a small number of specific plants as the main food resource, and tree canopy for feeding and sleeping sites, makes them vulnerable to changes in the environment. Further research, particularly on red colobus of West Africa which is understudied, is vital for the conservation of threatened with extinction red colobus (Linder et al. 2021).

4.2.5 Aims and predictions

The overarching goal of this chapter is to describe and evaluate the diet of western red colobus in two NPs in West Africa, with distinct anthropogenic characteristics. An updated and more detailed information on the diet of *P. b. badius* was obtained. Diets of populations from Gola RNP and Tiwai island (Davies et al. 1999), both in Sierra Leone, was compared. Additionally, the first dietary data on the subspecies *P. b. temminckii* of Guinea-Bissau was produced for a population living in Cantanhez NP. Spatial and temporal variation was evaluated for both NPs populations with the aim to provide information on the most important plants for the survival of endangered red colobus. The identification of plant species from non-invasive samples was achieved through the implementation of the molecular laboratory technique of DNA metabarcoding.

The specific goals of this research were:

(a) Characterise the diet and determine species richness of the two subspecies of *Piliocolobus badius*, the Bay colobus in Gola RNP (Sierra Leone) and Temminck’s red colobus in Cantanhez NP (Guinea-Bissau).
(b) Identify seasonal variation in the dietary composition of both populations and evaluate differences between males and females for *P. b. badius* individuals in Gola RNP.
(c) Investigate shifts in the diet of *P. b. temminckii* over time by comparing the same population in two periods, dry seasons of 2009 and 2019.

Predictions:

1. The diet of both subspecies will be diverse, with a small number of plants contributing the most to their diet. These are expected to refer to the most common food trees in each NP.
2. Intraspecific variation is expected to be observed in the diet of both primates across seasons as a result of food availability. Dietary differences between

sexes are not anticipated as there is no reference to such distinction in previous studies in colobine diet.

3. Temporal analysis in *P. b. temminckii* is likely to detect some intraspecific variation but no significant differences in species richness in diets 10 years apart, as the forest composition has not suffered major changes, nor reduced in size (Catarino et al. 2008; Catarino and Palminha 2018). Vegetation shifts may have taken place, but western red colobus are mobile species that can travel daily to feed on preferred items.
4. Lastly, crop foraging by red colobus of Cantanhez NP will be more evident than in the population of Gola RNP, as the forest in heavily fragmented and primates are more exposed to farmland.

4.3 Methods

4.3.1 Study sites

Non-invasive sampling was conducted in two National parks in two West African countries. Gola Rainforest National Park is an area of approximately 750km^2 of continuous forest in the southeast of Sierra Leone. The rainy season starts in May and ends in November, with July and August being the hottest months. Young foliage production peaks at the beginning of each season, with flowering occurring only in the early dry season, followed by the fruiting season. Despite there being two foliage peaks, young leaves are present throughout most year due to differences in leaf production from different plant species (Oates 1988). This largely intact rainforest is dominated by trees in the mature forest, with areas of secondary forest and regrowth in Gola south forest block and some in western Gola central. These result from old logging exploitation and the use of the forest by militias and local communities during the civil war. The most abundant plant families are Fabaceae and Euphorbiaceae, with *Heritiera utilis*, *Brachystegia leonensis* and *Cynometra leonensis* the most common trees (detailed information on forest types and common plants in Table A.1.1 in Appendix 1). The protected forest is absent from human settlements and has low levels of anthropogenic activities, mainly due to heavy conservation patrol and the presence of researchers. More than one hundred villages can be found in the community forest, an area 4 km wide surrounding the protected forest, which is highly populated and cultivated with crops. The park supports high levels of biodiversity, including several primate species, namely one of the few populations of Bay colobus (*Piliocolobus badius badius*).

Cantanhez, a National Park in the southwest of Guinea-Bissau, gazetted in 2008 is a peninsula of roughly 1,000km^2 of forest-savanna-mangrove-agricultural mosaic, densely populated by humans of diverse cultural groups. Its flora and fauna are diverse and Temminck’s red colobus (*Piliocolobus badius temminckii*) can be found mostly in the remaining small forest pockets in the area, located more often in the south of the park. The vegetation more common in the north of the park is grassland and woodland savanna. Its sub-humid forest is characterised by large trees such as *Ceiba pentandra*, *Parinari excelsa*, *Sterculiaa africana*, *Dialium guineense* and *Ficus* spp., under-story trees such as *Xylopia aethiopica*, and several liana species like *Hunteria elliotii* (Scott 1992; Rodrigues et al. unknown). A naturally occurring tree most commonly found in palm groves, *Elaeis guineensis*, is highly exploited by human communities. Some people have cash crop plantations of *Anacaridum occidentale* which has contributed to a small increase in vegetation cover in the park since the 1950s (Catarino and Palminha 2018). Cantanhez has a marked rainy season between June and November, with the other months being mostly dry. Young foliage has a peak in production in July and August, but new leaves are available annually depending on plant species. Flowering is most common in March, with ripe fruiting happening between February and May (Bessa 2014). Further details on the conservation management applied in the parks, and the demographics of resident populations can be found in Chapter 1, section 1.7.

4.3.2 Study species - Western red colobus*, Piliocolobus badius* (Kerr, 1792)

Old World monkeys (species of Africa and Asia) are usually grouped in one family, the Cercopithecidae, which consist of two distinct subfamilies, the Cercopithecinae and the Colobinae (Groves et al. 1993; Fleagle 2013). Colobines (leaf-eating monkeys) are an anatomically, ecologically, and an understudied socially variable primate group (Linder et al. 2021). The African colobines divide into three separate genera: *Piliocolobus* (red colobus), *Procolobus* (the olive colobus) and *Colobus* (black-and-white colobus) (Linder et al. 2021). The number of species in *Piliocolobus* (16-18 distinct taxa or “forms”) is not universally agreed mainly due to the possible hybridisation that occurs among a number of groups in the Democratic Republic of Congo (Grubb et al. 2003). Nevertheless, in this study we used the latest classification generated by the Primate Specialist Group of the IUCN Species Survival Commission (follows Groves 2007; Zinner et al. 2013). This reports 17 species, with *Piliocolobus badius* dividing into two subspecies (*P. b. badius and P. b. temminckii*).

This genus has had a complex evolutionary history distinguishing the 18 forms by coat colour and pattern, facial appearance, behaviour, size, genetics and vocalisations (Ting 2008; Linder et al. 2021). Red colobus can be found in sub-Saharan Africa, from Senegal and Gambia on the west coast to the Zanzibar archipelago in the east coast, but their distribution is patchy and they are mostly absent in equatorial Africa (Struhsaker 2010) (Fig. 4.2). Molecular data (Ting 2008) suggests a split into three main lineages during the Pliocene, which correspond approximately to the western, western equatorial and central equatorial/eastern assemblages (Cardini and Elton 2009).

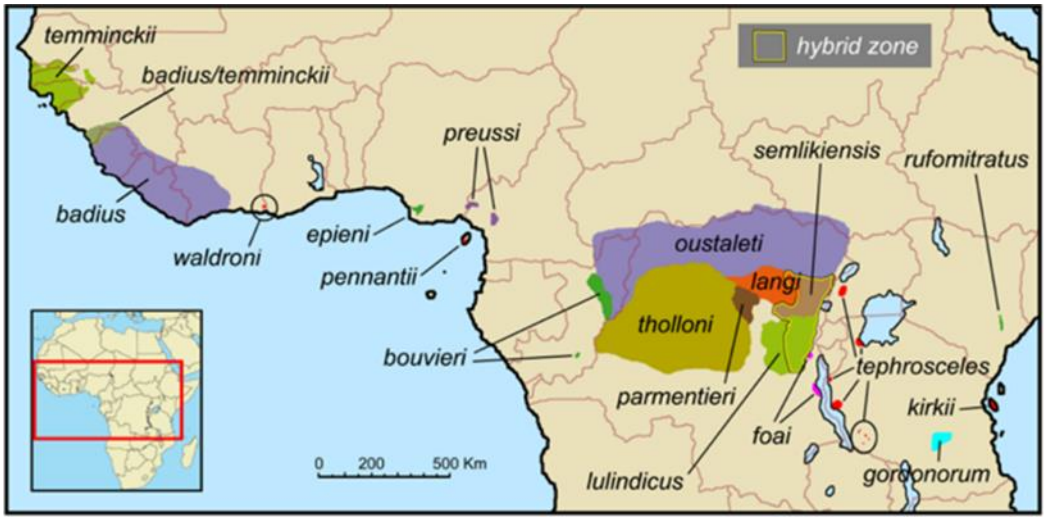


Figure 4.2 Spatial distribution of red colobus (*Piliocolobus*) and its latest distinct taxa (or "forms") agreed under the species Action Plan (map by Stephen D. Nash in Linder et al. 2021). The western red colobus (*Piliocolobus badius*) is represented by the subspecies Temminck's red colobus (*P. b. temminckii*) and the Bay colobus (*P. b. badius*) with green and purple distributions, respectively, on the left-hand side of the map.

The endangered western red colobus (*Piliocolobus badius*) occurs from Senegal to Côte d'Ivoire, classifying into two subspecies with a possible, yet unclear, boundary between the south of Guinea and the north of Sierra Leone (Harding 1984; Groves 2007; Oates and Nash 2011). Temminck's subspecies (*P. b. temminckii*) has been confirmed in Senegal, The Gambia, Guinea-Bissau and Guinea, while the Bay colobus (*P. b. badius*) is present in Guinea, Sierra Leone, Liberia and Côte d'Ivoire (McGraw et al. 2020; Minhós et al. 2020; Linder et al. 2021). The other species of the western lineage, *P. waldroni* previously recorded in Côte d'Ivoire and Ghana, is likely to be extinct (Oates et al. 2000).

Some research suggests that early colobines were partly terrestrial (Leakey et al. 2003), but the genus *Piliocolobus* is nowadays characterised as predominantly arboreal. This group occupies a variety of forest habitats including dry savanna woodland, lowland and medium altitude rainforest, montane forest, mangrove swamp and riparian forest (Struhsaker 2010). The genus' adaptation to the forest canopy places the group in a vulnerable position as most of its habitat is threatened by habitat loss, degradation and fragmentation due to global and local demand for natural resources (IUCN Red List). Logging, mining, and forest conversion to plantations, added to commercial and subsistence hunting is threatening every form of red colobus monkey with extinction (Linder et al. 2021). Red colobus are preferred species among hunters because they are a medium-sized primate restricted to high canopy strata, their detection in the forest is easier due to their large and vocal groups, and they deliver high rewards per unit of hunting effort (Refisch and Koné 2005; Oates and Nash 2011).

Red colobus monkeys weigh between 5.5-11.0 kg and they have long tails and hind feet to facilitate arboreality and leaping between trees (Struhsaker 2010). Most forms live in large multi-male, multi-female groups typically between 25 and 40 individuals (Davies and Oates 1994). Females are often present in higher numbers and are usually the dispersing sex (Starin 1994), an uncommon characteristic among primates which likely weakens female bonding within social groups (Davies and Oates 1994). Red colobus spend most of their days feeding or resting, and are frequently observed in association with other monkey species as a predator-avoidance strategy (e.g. with black-and-white colobus Guinea-Bissau (Minhós et al. 2013), and with Diana monkeys in Tai, Côte d'Ivoire (Holenweg et al. 1996; Kane and McGraw 2018) and Tiwai (Davies et al. 1999) and Gola RNP (pers. obs.), Sierra Leone).

Piliocolobus badius spp. badius (Kerr, 1792) - Gola Rainforest National Park, Sierra Leone

The Bay colobus (*P. b. badius*) inhabiting the forests of Sierra Leone (except in the northwest) has a darker coat which is a combination of black (face, crown, dorsum, tail) and red (ventrum and limbs) (Fig. 4.3). There are no overall population estimates for this subspecies but recent surveys in Côte d'Ivoire (Tai NP) (Galat and Galat-Luong 1985; Oliveira 2017), and in several parks and forest reserves of Liberia and Sierra Leone (Brncic et al. 2010; Foglietti 2020) reveal population decline and disappearance across much of its range. This subspecies IUCN Red List conservation status assessed in 2020, classified the taxa as endangered. Small and decreasing population may be linked to a range of threats, including hunting for subsistence of local human populations, for food markets in urban centres, and for workers in mining and logging camps in and around the forests (Furnell et al. 2015; Greengrass 2016). Deforestation, as a consequence of natural resource exploitation, large-scale industrial agriculture and infrastructure development due to population growth, added to the recent civil conflicts that took place in three of the four countries of the species distribution, are a continuous pressure to Bay colobus populations (Linder et al. 2021).

Estimates for *P. b. badius* in the last survey in Gola RNP (Klop et al. 2008) suggested a total population of 14,830 individuals and a population density of 19.8 ind./km^2 (95% CI = 11.4 – 34.1). This density is extremely low (density ranges from 18ind./km^2,Chapman and Chapman (1999); to 313 ind./km^2, (Chapman et al. 2002), especially when compared with populations of *P. tephrosceles* living in a forest with similar size area (e.g. Kibale, 766km^2, 176 ind./km^2) (Struhsaker 2010). The distribution of western red colobus was recorded across all forest blocks (Gola south, Gola central and Gola north) with group densities almost twice as high in Gola north, exhibiting a preference for primary forest. However, a much lower estimate has been reported in the red colobus action plan for the same site (5,000 individuals, Linder et al. 2021). No dietary studies have been conducted on *P. b. badius* in Gola RNP, but scan sampling of habituated populations of this subspecies were conducted in the protected area of Tiwai island, in the southwest of Gola RNP (see Fig. GRNP) (Davies et al. 1999). Seeds, young leaves, and mature leaf parts contributed to 73% of the species diet, complementing it with a high intake of whole fruits and flowers. Nearly half of the annual diet consisted of non-foliar items. The red colobus conservation action plan for 2021-2026 proposes species surveys to assess presence/absence, relative abundance and threats, as well as implementation or improvement of enforcement laws for the protection and conservation of the subspecies.

Piliocolobus badius spp. temminckii (Kuhl, 1820) - Cantanhez National Park, Guinea-Bissau

Temminck's red colobus is the westernmost distributed of all *Piliocolobus* and its coat is lighter with grey colour on the face, back, tail and crown, and orange limbs and lighter on the ventrum (Fig. 4.3). As with *P. b. badius*, there are no population size estimates for *P. b. temminckii* subspecies, but numbers are low (2,500 individuals across the whole distribution range). This endangered species occurs in small isolated forests, with five relatively large populations, including Cantanhez NP, and the remaining known populations not exceeding 60 individuals (Minhós et al. 2020b and references there in). Research on Temminck's red colobus in Senegal (Galat-Luong and Galat 2005), Guinea-Bissau (Minhós et al. 2016; Bersacola et al. 2018) and The Gambia (Mayhew et al. 2020) indicate population decline, isolation and displacement. Most remaining populations live in very small pockets of protected forest (< 6km^2) surrounded by settlements, roads, farms and other human infrastructures. Even in larger protected areas, forest fragmentation is a main concern and red colobus survive in restricted patches of undisturbed habitat (Linder et al. 2021). Threats to this 'Endangered' species (IUCN Red List conservation status) vary by location but are similar to those affecting *P.b. badius*. However, *P. b. temminckii* is also threatened by infectious diseases transferred from humans and domesticated animals (e.g. yaws), habitat reduction due to human-induced fires and decrease in rainfall (a result of anthropogenic factors and climate change), and consequently, increased exposure to predators (pythons, hyenas, dogs and humans) (Galat-Luong and Galat 2005; Hillyer et al. 2015; Düx et al. 2017).

The current effective population size of *P. b. temminckii* in the Cantanhez NP is estimated to be between 251 and 575 (Minhós et al. 2016). This reflects a population decline of one order of magnitude c. 1,500-3,500 years ago (with 90% highest posterior density (HPD90%) intervals between 27 and 52,481). At the time, the ancestral effective population size was estimated at 13,490 - 15,136 (HPD90% intervals ranging from 3,540 to 60,256), but red colobus may be restricted to the southern part of the park. Dietary studies of *P. b. temminckii* are absent for populations in the NP. Population surveys and specific conservation approaches such as the implementation of monitoring programs, reforestation actions, training and hiring of forest guards and patrols, and the creation of infrastructures on the limits of NPs are some of the strategies proposed by the red colobus Action Plan.

Figure 4.3 Coloration differences between the two subspecies of western red colobus. On the left is *P. b. badius* in Gola RNP, Sierra Leone, and on the right is *P. b. temminckii* present in Cantanhez NP, Guinea-Bissau feeding on the fruits of *Parkia biglobosa*.

4.3.3 Field and Laboratory data

This highly threatened group of primates lives in habitats which are often not of easy access (e.g. due to geographic characteristics or political conflict), are usually elusive and are not habituated to human presence mainly due to hunting pressure. They disperse far and fast through their home ranges, and many species feed high up in the canopy. These characteristics pose a difficulty for following groups and carrying out direct observations, and consequently restrict the number of samples collected and sites studied. Morphological examination of faeces is another option to study the diet of primates, but it is time demanding and many items are easily digested resulting in an altered appearance which makes the identification process difficult. Thus, the technique that appeared the most adequate for this study was DNA metabarcoding of non-invasive faecal samples. Primates are not the only group where diet is problematic to study, and in the past two decades, DNA metabarcoding has been a successful alternative to more traditional approaches across many taxa. In some cases, the dietary diversity of elusive species has been described or expanded, and for many, rare prey items have been revealed (Ait Baamrane et al. 2012; Lyke et al. 2019; Schmack et al. 2021; Querejeta et al. 2022). DNA metabarcoding uses universal primers to amplify short DNA fragments of the species of interest. In this case, the ITS2 universal primer was used to amplify the DNA of plants present in the faecal matter of primates. Some information on the methodology is presented below, but for a more detailed, please refer to Chapter 1, section 3.3.3.

Samples collected in the field (Gola RNP, N = 402; Cantanhez NP = 338) belonged to many different primates present in the NPs, including western chimpanzees. After barcoding for taxonomic identification using mitochondrial markers, samples resulted in 231 *P. b. badius* samples from Gola RNP, and 121 *P. b. temminckii* from Cantanhez NP. Sample collection location can be observed in maps Fig. 3.3 and Fig. 3.4 in Chapter 3, section 3.3.3.1. A total of 352 western red colobus DNA samples were selected to go through the DNA metabarcoding process. In addition, 60 DNA samples of Temminck's red colobus collected in a previous work conducted in Cantanhez NP during the dry season of 2009, were added to the process (see Fig. A.4.1 in Appendix 4 for sample location). These samples had the same collection and extraction methods implemented in the current project, but a different preparation prior to storage was used. The preparation step involved immersing fresh samples in > 96% ethanol for 24-48 hours, and then desiccating and storing them in silica (more information available in Minhós 2012). Information obtained from these samples allow temporal analysis for the *P. b. temminckii* subspecies. In total, 412 samples from both red colobus taxa from all years, were used in the initial phase of the DNA metabarcoding process (amplification with UniPlant markers, below).

Details on the PCR conditions using the UniPlant primers can be found in Chapter 3, section 3.3.3.3. Some samples were excluded from the HTS step because they failed to amplify in the UniPlant PCR (N = 38 *P. badius*). This step had 90.8% amplification success. The remaining 374 samples (Gola RNP 2018: *P.b. badius* = 212; Cantanhez NP 2009/2019: *P.b. temminckii* = 162) were amplified with MID-tag primers, and sent to HTS. Bioinformatic analysis is explained in Chapter 3, section 3.3.3.3. The Illumina sequencing and subsequent cleaning stages produced a dataset with 368 red colobus samples with good quality plant reads, five samples with zero plant reads, and one sample that showed some evidence of contamination. The six samples with no results or contamination were excluded from the final dataset.

4.3.4 Statistical analysis

A final dataset comprising 368 western red colobus samples (Gola RNP, N = 207; Cantanhez NP: 2009, N = 60; 2019, N = 101) was obtained. Most analyses presented in this chapter are identical to those performed in Chapter 2. In section 3.3.4, a description of the bioinformatics methods applied to the DNA metabarcoding results can be found, along with the statistical tests employed. All statistical analysis were conducted in R Statistical software v4.1.3 (*R Core Team* 2022) using mainly the packages *iNEXT* (Hsieh et al. 2016), *mvabund* (Wang et al. 2012), and *vegan* (Oksanen et al. 2019) and *ggplot2* (Wickham et al. 2016) for visualisation purposes.

Descriptive statistics are depicted for the western red colobus populations of each NP and compared with previous studies on the same species conducted in nearby study sites. The list of plant taxa detected in the diet of each subspecies and respective frequency of occurrence (F_o%) is detailed. Hill-diversity and sample coverage were estimated for both datasets to assess the efficiency of sampling to cover the dietary community of western red colobus. Multivariate generalised linear models (MGLMs) and Likelihood Ratio tests were used to evaluate the effects of seasonal food availability on the dietary composition of both subspecies. Dietary differences between 112 females and 42 males were tested for *P. b. badius*, and variation across time was investigated for *P. b. temminckii*. The dataset for sex variation is smaller because amplification of the sexing marker was not successful for 53 samples. Modelling between the areas of each park with distinct vegetation were attempted (e.g. Gola central and south; Cantanhez north and south). In Gola RNP, the south forest block was intensively logged and probably has more secondary forest. So an analysis of variance was performed to assess the variation in diet composition between the two forest blocks (Gola central = 180; Gola south = 27). In Cantanhez NP, however, due to only two samples being collected in the north of the park, this comparison was not possible.

4.4 Results

The western red colobus (*P. badius*) dataset included a total of 308 samples from the two NPs, plus 60 samples collected in 2009 to perform a ten-year gap analysis in Cantanhez NP. Of the initial 6,967,262 reads obtained across the three SP, 1.7% were identified as reads with sequencing errors or chimeras, and were removed from the dataset. An average of 7,147 DNA reads (±3,593 SE) were obtained per sample, and a total of 14,867 zOTUs were compared with the NCBI GenBank reference database. Several zOTUs were discarded due to no or unsure taxa assignment (9.9%), taxa not native to or introduced in West Africa (1.1%), assigned to bacterial taxa (0.2%) and to fungi (3.4%). This last percentage is relatively high possibly because the marker is also a universal barcode for fungi (Schoch et al. 2012) and some samples had developed fungi during the storage period. During the DNA metabarcoding sample preparation, control negatives, positives and blanks were added to address contamination issues and possible MID-tag jumping during the HTS. We did not detect tag jumping in the MID-tag combinations used in this thesis, but this issue had been previously studied by other researchers in the same lab, that had produced similar DNA metabarcoding projects and indicated which MID-tag combinations should not be used. The maximum contamination detected across the three controls was low (1.0 %), with 4.0 % for negative controls, 0.3 % for blank samples, and 2.2% for positive controls. The combined read maximum count originating from contamination were removed from each DNA sample, producing a dataset with the same number of taxa (zOTU) as the initial dataset (228), but with a much lower count number per zOTU (82.8% reduction in count number per zOTU). Additionally, in order to have a conservative approach to possible contamination, a 1% threshold per zOTU was applied to eliminate low read counts. This step reduced the number of final zOTU detected in the diet of *P. badius and P. t. verus*. A final 8,753 dietary presence counts were found in western red colobus and chimpanzee samples across the three years. These corresponded to 171 plant taxa identified to species or genus level, belonging to 45 plant families.

Western red colobus only, revealed 97 plant taxa in their diet across the years (91 taxa, 37 families in 2018 and 2019 data only). A total of 64 plant species and 33 genera, belonging to 40 families, were identified. An average of 4.48 plants (±SE 1.84) were detected in each sample with a minimum of one and a maximum of 11 taxa per sample. One fifth of plant taxa were detected in one single sample but was still included in the analysis as this is a result obtained after cleaning the dataset for contamination. All plants in the diet of western red colobus, detected using DNA metabarcoding, were recorded in the botanical surveys conducted in each NP or have been recorded in the study site countries.

4.4.1 Dietary richness

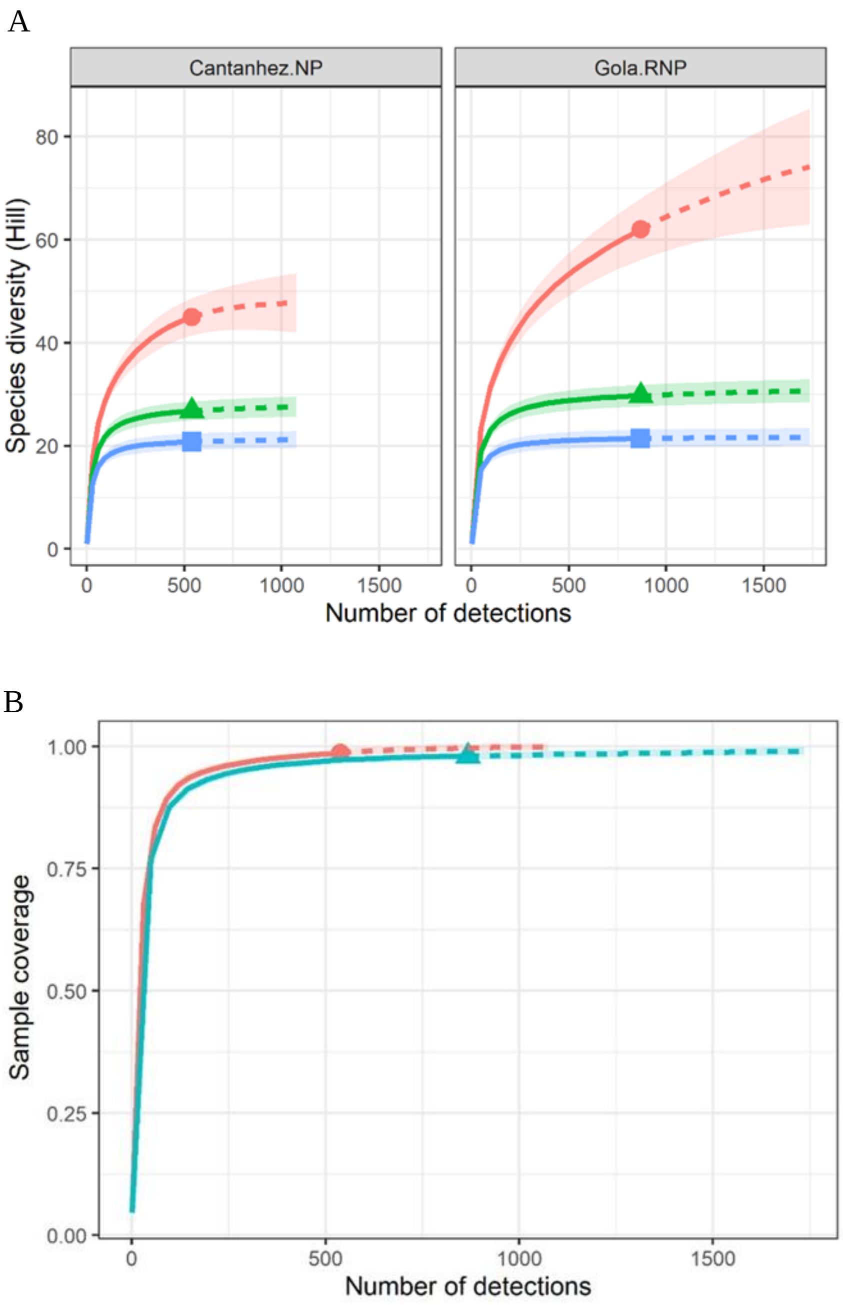
Three subsets were obtained for the species: Gola RNP, N = 207 faecal samples collected over five months; Cantanhez NP: in 2019, N = 101 faecal samples collected over three months; in 2009, N = 60 faecal samples collected over 7 months. Just over 7% of western red colobus diet across study sites and years is constituted by crops. The diet of the subspecies *P. b. badius* in Gola RNP and *P. b. temminckii* in

Cantanhez NP, varied in the raw count of plant taxa and average number of plants detected per sample (Table 4.1). This variation was evaluated across season and time, and sex differences were also examined (sections below).

Table 4.1 Western red colobus plant dietary richness. Number of plant taxa detected in the diet of *P. b. badius* and *P. temminckii* in West Africa. Plant richness per sample is shown for each NP population, and per season. Total sample size of per NP as in brackets per season (D = dry; R = rainy). % Crops: the number of plants detected in red colobus diet that is a cultivated or introduced item, and in brackets the total frequency of occurrence (TF_o%) of such items in red colobus faeces. The dataset from Cantanhez NP 2009 only refers to the dry season, as no samples were collected in the rainy season.

			Per sample					Per study site		
					Plant richness (±SE)			% Crops (TF_o%)		
National park	Sample size (D/R)	No. plant taxa	Min.	Max.	Both	Dry season	Rainy season	Both	Dry season	Rainy season
Gola	207 (90/117)	62	1	9	4.19 (±1.7)	4.23 (±1.7)	4.16 (±1.7)	3.2 (1.4)	5.7 (4.4)	3.6 (1.7)
Cantanhez 2019	101 (68/33)	45	1	11	5.33 (±1.9)	4.69 (±1.6)	6.64 (±1.8)	11.1 (16.8)	10.8 (20.6)	10.7 (9.1)
Cantanhez 2009	60 (60/0)	36	1	8	4.05 (±1.9)	4.05 (±1.9)	-	5.6 (33.3)	5.6 (33.3)	-

Overall, Hill-diversity estimates suggest that sampling conducted in both NPs was sufficient to cover a large majority of the dietary community (Fig. 4.4, panel B). Only less than 5% of the dietary taxa was not detected for each sample subset (dietary coverage ±95% CI: Gola RNP = 98.1 ±0.9; Cantanhez NP 2019 = 98.6 ±0.9; Cantanhez NP 2009 = 96.3 ±1.8). The Hill-richness exponent (l = 1) provided higher diversity estimates than the other two exponents (Hill-Shannon, l = 0; Hill-Simpson, l = -1), which reach an asymptotic result at the same number of detections (read counts). The result suggests that the dietary community of western red colobus is composed of many rarely consumed species as exponent $_{l}$ = 1 is more sensitive to rare species (Fig. 4.4, panel A, and Fig. A.4.1).



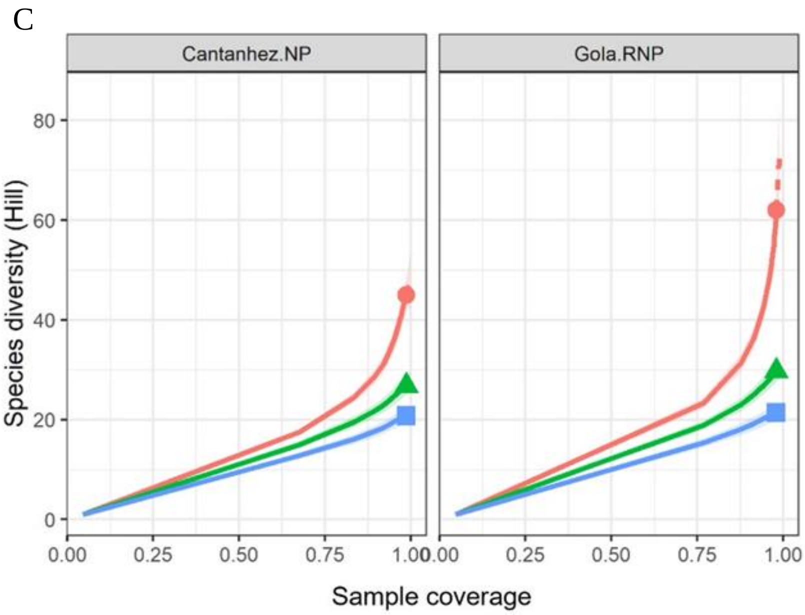


Figure 4.4 Western red colobus dietary species diversity and community coverage. The percentage of plant species detected in western red colobus non-invasive samples using a DNA metabarcoding approach are estimated with three exponents with different sensitivities to species rarely consumed by *P. b. badius*. Values of the exponent l, which determine the rarity scale of different diversity estimates, are represented in different colours: Hill-richness ($l = 1$), red line with circle; Hill-Shannon ($l = 0$), green line with triangle; Hill-Simpson ($l = -1$), blue line with square. Solid lines are the observed diversity, which is further extrapolated (dashed lines). Shading around the line correspond to 95% confidence intervals. Top image (A) shows the species diversity observed for the total number of dietary detections in red colobus populations of Cantanhez NP (left) and Gola RNP (right). Middle graph (B) demonstrates how much of the dietary community was possible to cover in Cantanhez NP (red line with circle) and Gola RNP (blue line with triangle), with the number of detections resultant from the DNA metabarcoding. Bottom image (C) illustrates the species richness per sample coverage in Cantanhez NP (left) and Gola RNP (right).

More than half of the plant taxa detected in this study, were recorded as food items in previous red colobus research (Table A.4.1 in Appendix 4). These represent plants with the highest frequency of occurrence in this study. Those taxa for which information was not available in the literature, had lower presence in western red colobus samples (on average, present in 3 samples only out of 368).

P. b. badius in Gola RNP

The number of plant families in *P. b. badius* diet was higher than for *P. b temminckii* across both years (Gola = 30 families; Cantanhez 2019 = 24; Cantanhez 2009 = 20). The plant families which were present in 50% of Bay colobus samples in Gola RNP were Fabaceae (18.4%), one of the most common plant families in the park, Combretaceae (12.7%), Rubiaceae (9.6%) and Chrysobalanaceae (9.2%) (Fig.4.5). The 'other' group accounts for 16 families, each present in less than 1% of the samples. This includes Urticaceae, Malvaceae and Apocynaceae families among others.

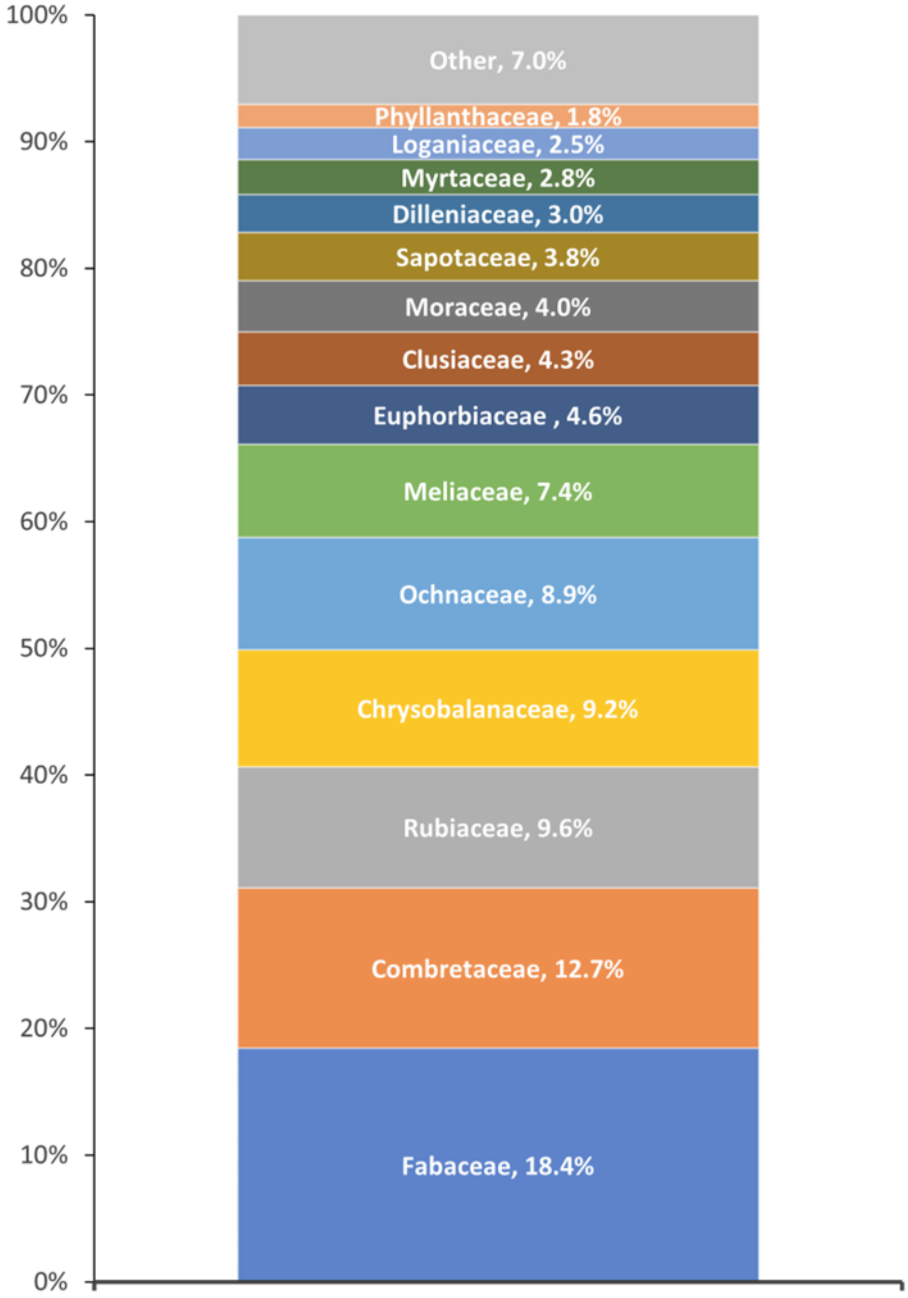


Figure 4.5 Gola RNP red colobus dietary composition by plant family. The frequency of occurrence of plants detected in *P. b. badius* diet per plant family was calculated using dietary counts. Six families contributed to around 70% of the dietary requirements of this primate species in Gola RNP. For visualisation purposes, 16 additional plant families were grouped together under the category ‘Other’. These taxa were detected in 1% or less of all 207 samples.

A total of 62 plant taxa were present in the diet of *P. b. badius*, but four taxa were the most frequently detected ($F_o > 30\%$), and common across seasons. The top species are *Lophira alata*, the only member of the Ochnaceae family, *Anthonota macrophylla*, *Combretum* spp. and *Maranthes aubrevillei* (Table 4.2; full list in Table A.4.2 in Appendix 4). The top 20 plant taxa were all present in samples collected in the dry and rainy seasons, except *Nauclea* spp. which was only detected in samples collected in the rainy season. These plant genera and species were mostly trees and shrubs, with vines and climbers being detected in fewer samples. One introduced plant (*Enterolobium* spp.) and two native plants exploited by humans in Sierra Leone (*Cola* spp. and *Coffea* spp.) were detected, but they were rarely present across samples ($F_o < 2.5\%$). Despite being native, Kola trees are sometimes grown as crops by local villagers. However, as in this study most sampling took place in the protected forest, *Cola* spp. will not be considered a crop. Hence, crops *Enterolobium* spp. and *Coffea* spp. accounted for 3.2% of *P. b. badius* diet in Gola RNP. Both items were eaten during the dry and rainy season, but *Coffea* spp. had a higher frequency of consumption in the dry season, probably because that is when the unripe fruit is available.

Table 4.2 Top 20 plant taxa detected in Bay colobus diet at Gola RNP. Characteristics of wild and crop plant taxa detected in *P. b. badius* non-invasive samples using a DNA metabarcoding approach. Life form: climber (C), shrub (S), tree (T). Season: dry (D) Dec-Apr; rainy (R) May-Nov. F_o(%): Frequency of occurrence, the number of times plant taxa was detected across samples (N = 207). Part eaten: flower (Fl), fruit (Fr, mostly unripe), mature leaves (ML), young leaves (YL), seeds (S); this information was obtained from previous studies (references in Table A.4.1 in Appendix 4). Status: IUCN Red List Conservation status, least concerned (LC), near threatened (NT), vulnerable (VU), not available (NA). For a full list of the 62 plants consumed by Bay colobus, please see Table A.4.2 in Appendix 4.

Plant family	Dietary taxon	Life form	Season	F_o(%)	Plant part eaten	Status
Ochnaceae	*Lophira alata*	T	D, R	37.20	-	VU
Fabaceae	*Anthonotha* spp.	T	D, R	36.71	YL	LC
Combretaceae	*Combretum* spp.	S	D, R	33.82	Fl, ML, S, YL	LC
Chrysobalanaceae	*Maranthes aubrevillei*	T	D, R	33.82	-	NA
Rubiaceae	*Nauclea* spp.	T	R	21.26	Fl, YL	NT, LC
Clusiaceae	*Pentadesma butyracea*	T	D, R	17.39	-	LC
Moraceae	*Ficus* spp.	T	D, R	16.91	Fr, ML, YL	LC
Fabaceae	*Didelotia idae*	T	D, R	15.94	-	NT
Euphorbiaceae	*Manniophyton fulvum*	S, C	D, R	15.46	ML, YL	NA
Rubiaceae	*Keetia* spp.	T, S	D, R	14.98	-	LC
Meliaceae	*Carapa procera*	T	D, R	14.49	-	LC
Sapotaceae	*Manilkara obovata*	T	D, R	13.53	-	LC
Dilleniaceae	*Tetracera* spp.	T	D, R	12.56	-	NA
Meliaceae	*Trichilia monadelpha*	T, S	D, R	12.08	Fl, ML, YL	LC
Myrtaceae	*Eugenia* spp.	T, S	D, R	11.59	-	LC
Combretaceae	*Strephonema pseudocola*	S, T	D, R	11.11	-	LC
Loganiaceae	*Strychnos* spp.	S, T	D, R	10.63	Fl, YL	NA
Combretaceae	*Terminalia* spp.	T, S	D, R	8.21	Fl, S, YL	VU
Phyllanthaceae	*Bridelia micrantha*	T, S	D, R	7.73	YL	LC
Fabaceae	*Cryptosepalum tetraphyllum*	T	D	7.25	-	VU
Crops						
Rubiaceae	*Coffea* spp.	S, T	D, R	1.93	-	LC
Fabaceae	*Enterolobium* spp.	T	D, R	0.97	-	LC

P. b. temminckii in Cantanhez NP

Across 538 zOTU detections obtained from the 101 faecal samples of *P. b. temminckii* collected in 2019, it was possible to identify 45 plant taxa. These belonged to 24 families, with species from Fabaceae, Moraceae and Rubiaceae contributing to nearly 50% of the primate diet (Fig. 4.6). *Treculia africana* was detected in more than half of the samples, and four other species were present in more than 35-40% faecal samples (Table 4.3). The type of plants identified as the most important in the diet of *P. b. temminckii* was varied, including trees, shrubs, climbers and vines.

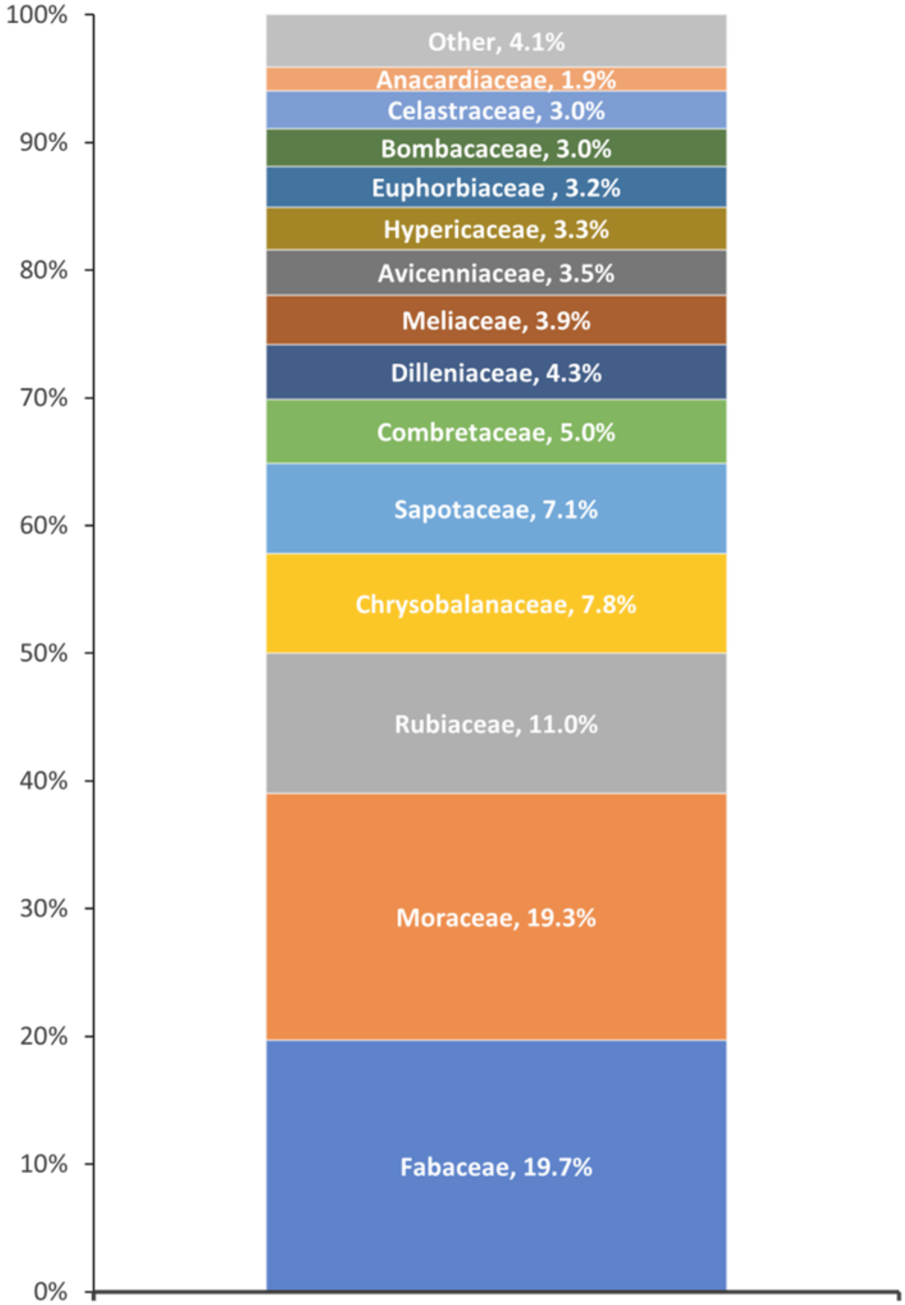


Figure 4.6 Cantanhez NP red colobus dietary composition in 2019 by plant family. The frequency of occurrence of plants detected in *P. b. temminckii* diet per plant family was calculated using dietary counts. Three families contributing to 50% of the dietary requirements of this primate species in Catanhez NP. For visualisation purposes, nine additional plant families were grouped together under the category ‘Other’. These taxa were detected in 1% or less of all 207 samples. The data presented here refers to the 2019 dataset. For an analysis on the 2009 dataset, please refer to Fig. A.4.2 in Appendix 4.

The top 20 plants with highest frequency were all native to Guinea-Bissau. Most plants were detected in samples collected in the rainy and dry seasons, with a few being only present in one of the seasons (e.g. *Uncaria africana*, *Ceiba pentandra*). This is likely a reflection of the folivorous diet of red colobus, which can easily find young and mature leaves throughout the year. A high number of crops were identified in the dietary composition of Teminck's red colobus (11%). However, they all had low frequencies of occurrence: mango (8.9%), coffee (4%), *Solanum* spp. (2%), cashew (1%) and *Cucumis* spp. (1%). The presence of *Coffea* spp. in Cantanhez NP samples was unexpected because, though native to Guinea-Bissau, the plant has not been recorded in any of the park botanical surveys nor reported as cultivated in the region. This result could be a contamination but comparisons with Gola RNP suggest otherwise. The same number of dietary counts was detected in samples from both NPs (N = 4), and the frequency of occurrence was higher in Cantanhez NP than in Gola RNP, where the plant is grown as a crop. Moreover, samples containing this genus were collected in both seasons, and in Gola, in different transects. This outcome requires further analysis to understand if *P. badius* are truly consuming coffee plant parts at both sites (e.g. amplify DNA samples individually with a primer specific to *Coffea* spp.), but for this research, *Coffea* spp. will not be excluded from either datasets.

Table 4.3 Top 20 plant taxa detected in Temminck’s red colobus diet at Cantanhez NP. Characteristics of wild and crop plant taxa detected in *P. b. temminckii* non-invasive samples in 2019 using a DNA metabarcoding approach. **Life form**: climber (C), shrub (S), tree (T), vine (V). **Season**: dry (D) Dec-May; rainy (R) Jun-Nov. **F_o(%)**: Frequency of occurrence, the number of times plant taxa was detected across samples (N = 101). **Part eaten**: flower (Fl), fruit (Fr, mostly unripe), mature leaves (ML), young leaves (YL), seeds (S); this information was obtained from previous studies (references in Table A.4.1 in Appendix 4). **Status**: IUCN Red List Conservation status, least concerned (LC), vulnerable (VU), not available (NA). For a full list of the 45 plants consumed by Temminck’s red colobus, please see Table A.4.3 in Appendix 4. For information on the diet of *P. b. temminckii* in 2009, please refer to Table A.4.4 in Appendix 4.

Plant family	Dietary taxon	Life form	Season	F_o(%)	Plant part eaten	Status
Moraceae	*Treculia africana*	T	D, R	51.49	ML, YL	LC
Fabaceae	*Leptoderris* spp.	C	D, R	39.60	-	LC
Chrysobalanaceae	*Parinari excelsa*	T	D, R	39.60	Fl, Fr, ML, YL	LC
Moraceae	*Ficus* spp.	T	D, R	38.61	Fr, ML, YL	LC
Sapotaceae	*Malacantha alnifolia*	S, T	D, R	34.65	-	NA
Combretaceae	*Combretum* spp.	S	D, R	26.73	Fl, ML, S, YL	LC
Rubiaceae	*Ixora* spp.	S	D, R	25.74	-	LC
Fabaceae	*Cassia sieberiana*	T, S	D, R	24.75	-	LC
Dilleniaceae	*Tetracera potatoria*	T	D, R	22.77	-	NA
Avicenniaceae	*Avicennia germinans*	T, S	D, R	18.81	-	LC
Hypericaceae	*Harungana madagascariensis*	T, S	D, R	17.82	-	LC
Rubiaceae	*Uncaria africana*	S	R	16.83	-	NA
Bombacaceae	*Ceiba pentandra*	T	D	15.84	-	LC
Fabaceae	*Mezoneuron* spp.	V	D, R	14.85	-	NA
Celastraceae	*Simicratea welwitschii*	C	D	13.86	-	NA
Fabaceae	*Dichrostachys cinerea*	T, S	R	12.87	Fl, ML, YL	LC
Moraceae	*Milicia regia*	T	D, R	12.87	-	VU
Euphorbiaceae	*Phyllanthus muellerianus*	S, V	D, R	12.87	ML, YL	NA
Meliaceae	*Trichilia prieureana*	T	D	12.87	Fl, ML, YL	LC
Fabaceae	*Crudia senegalensis*	S	R	8.91	-	NA
Crops						
Anacardiaceae	*Mangifera indica*	T	D	8.91	-	DD
Rubiaceae	*Coffea* spp.	S, T	D, R	3.96	-	LC

Solanaceae	*Solanum* spp.	H	D, R	1.98	-	NA
Anacardiaceae	*Anacardium occidentale*	T	D	0.99	-	LC
Cucubitaceae	*Cucumis* spp.	H	R	0.99	-	LC

4.4.2 Spatial variation: season and sex

Differences in the number of plants (f = 4.06, m = 4.4; W = 1966, *p*-value > 0.05) or the type of plants ingested (LRT = 5.66, *p*-value = 0.134) by female and male red colobus in Gola RNP were not significant (as prediction 2). The overlap between female and male diets is clear in the NMDS plot (Fig. 4.7).

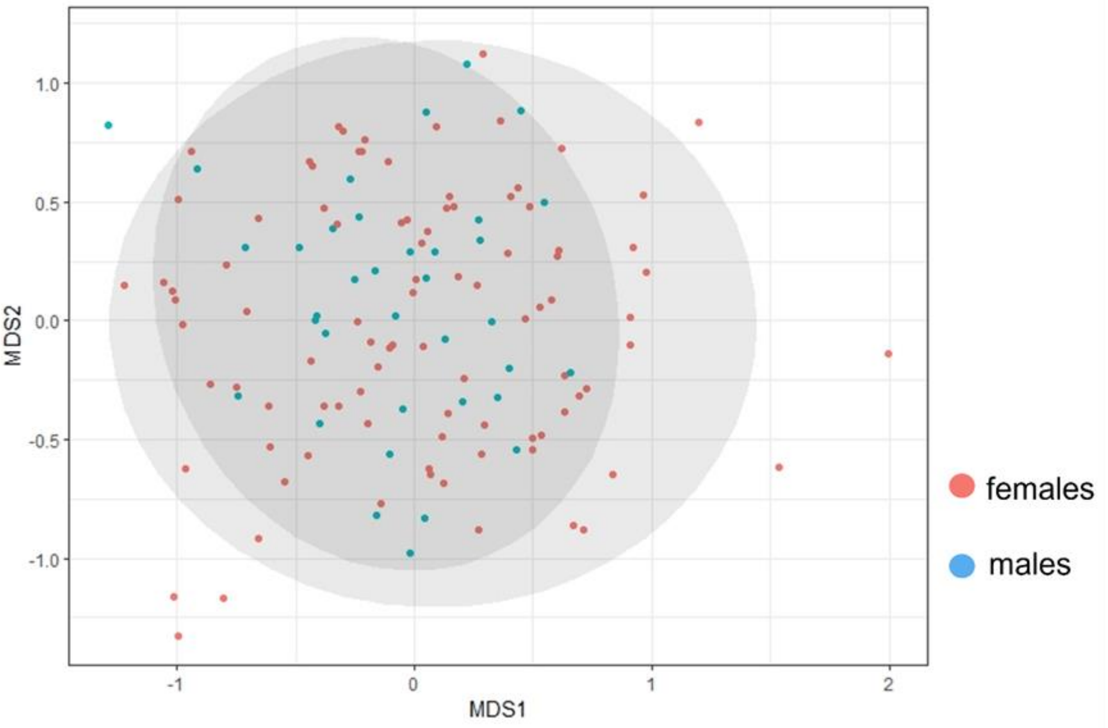


Figure 4.7 Sex dietary variation in Bay colobus of Gola RNP. Pairwise biplots from non-metric multidimensional scaling (NMDS) analysis represent a non-significant variation in dietary composition of female (red dots, wider elipse) and male red colobus (blue dots, narrower elipse). Molecular sex identification was not achieved for all samples; hence, 170 samples were used in this analysis: females = 120, males = 50.

Average plant richness per sample was similar between seasons in Gola RNP (dry = 4.23, rainy = 4.16; W = 5299.5, *p*-value > 0.05), but the diet composition in the dry and rainy seasons varied significantly (LRT = 328.1, *p*-value = 0.001). This variation is visualised in the NMDS and ggplot2 figure (Fig. 4.8). Ten plants contributed to the seasonal variation (significant GLM results) with the most significant being *Cryptosepalum tetraphyllum* (LRT = 26.52) only consumed in the dry season, *Nauclea* spp. (LRT = 59.24) fed on in the rainy season only, and *Eugenia* spp. (LRT = 34.71) and *Trichilla monadelpha* (Wald = 22.82) present in samples collected in both seasons (*p*-value = 0.001 for all taxa). Red colobus in other study sites have been recorded eating young leaves and flowers of *Nauclea* species, and in Gola, this genus flowers at the beginning of the rainy season which would explain the result.

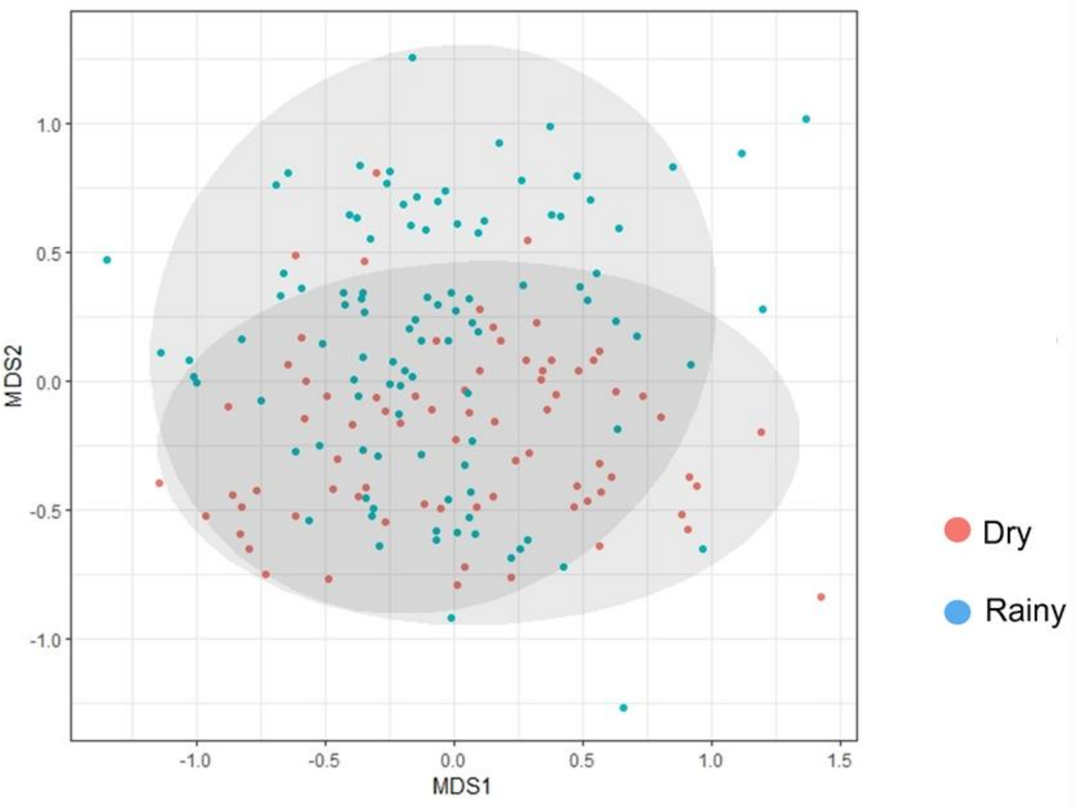


Figure 4.8 Seasonal dietary variation in Bay colobus of Gola RNP. Pairwise biplots from non-metric multidimensional scaling (NMDS) analysis represent a significant

variation in dietary composition of *P. b. badius* (N = 207) between the dry season (red dots, lower elipse) and rainy season (blue dots, top elipse).

In Cantanhez NP 2019, the mean number of plant taxa identified per sample in each season differed significantly (W = 480.5, *p*-value < 0.001). For this dataset, information on the sex of the individual samples was not available due to lack of resources and time, so only seasonal variation was evaluated. The MGLM model revealed that the diet composition in the dry and rainy seasons were significantly different (LRT = 398.6, *p*-value = 0.001), clearly visualised in Fig. 4.9. Six of the twelve plants that had a significant effect on seasonal variation (*p*-value < 0.05) were consumed in both seasons, but three were only eaten in the dry season and the remaining three in the rainy season. Without phenology data it is difficult to explain why these plants contributed strongly to the variation observed, as other red colobus populations were recorded feeding on many plants parts of these plants (data not available for half of the taxa, though). However, *Uncaria africana* (LRT = 45.83), *Dichrostachys cinerea* (LRT = 33.30) and *Crudia* spp. (LRT = 22.02) (all p-value = 0.001) were plant taxa only detected in samples collected in the rainy season and in Catombói forest. This could suggest that plant distribution in the different habitats in the Cantanhez could determine seasonal variation, but these three plants are found in all habitat types.

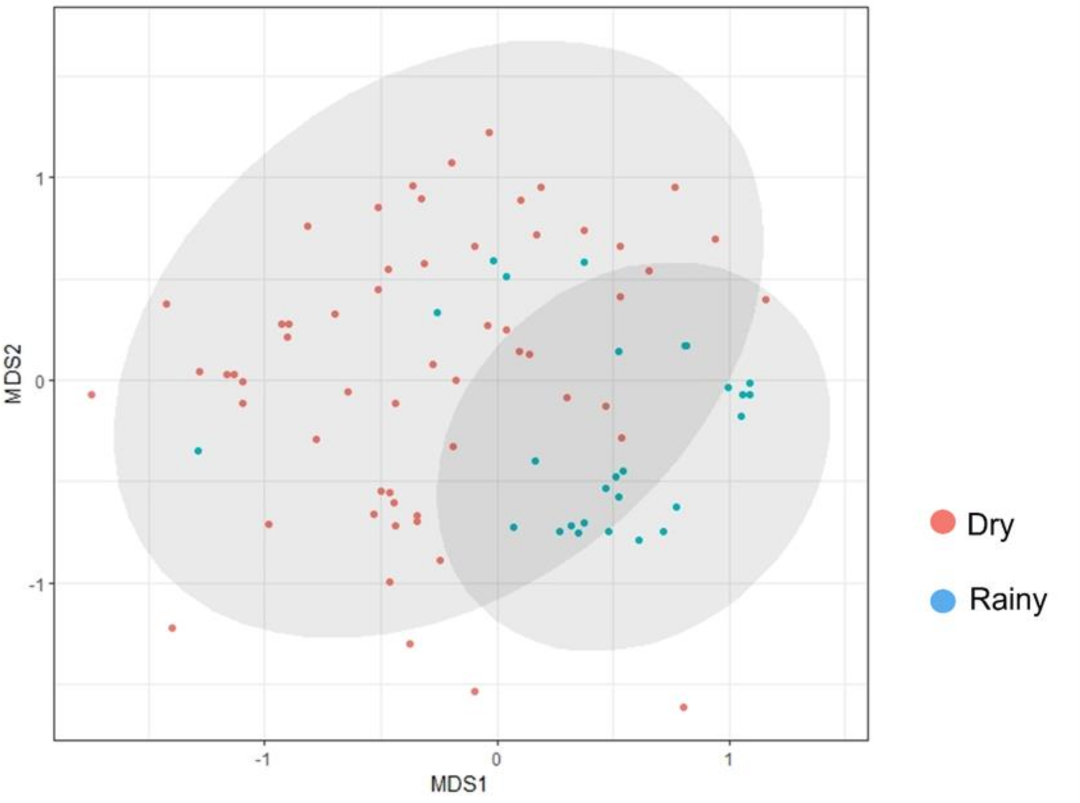


Figure 4.9 Seasonal dietary variation in Temminck’s red colobus of Cantanhez NP. Pairwise biplots from non-metric multidimensional scaling (NMDS) analysis represent a highly significant variation in dietary composition of *P. b. temminckii* (N = 101) between the dry season (red dots, left elipse) and rainy season (blue dots, right elipse).

4.4.3 Temporal and spatial variation in western red colobus

The Temminck’s red colobus population of Cantanhez NP was sampled with a ten-year gap. In the year of 2009, non-invasive samples (N = 60) were collected during the dry season. For comparison purposes, we created a subset of Cantanhez 2019 samples collected in the dry season only (N = 68). Raw counts of consumption in the diet reveal that the total number of plant taxa in both datasets was similar (2009 = 36 taxa; 2019 = 37 taxa) and the mean number of plants in each sample between years was not significantly different either (W = 2425, p-value = 0.06). Moreover, *P. b. temminckii* diet in 2009 and 2019 overlap (Fig. 4.10), but the composition varied significantly (LRT = 173.3, p-value = 0.001) mainly due *Anisophyllea* spp. which was only consumed by red colobus in 2009, and *Tetracera* spp. which was only eaten by the species in 2019.

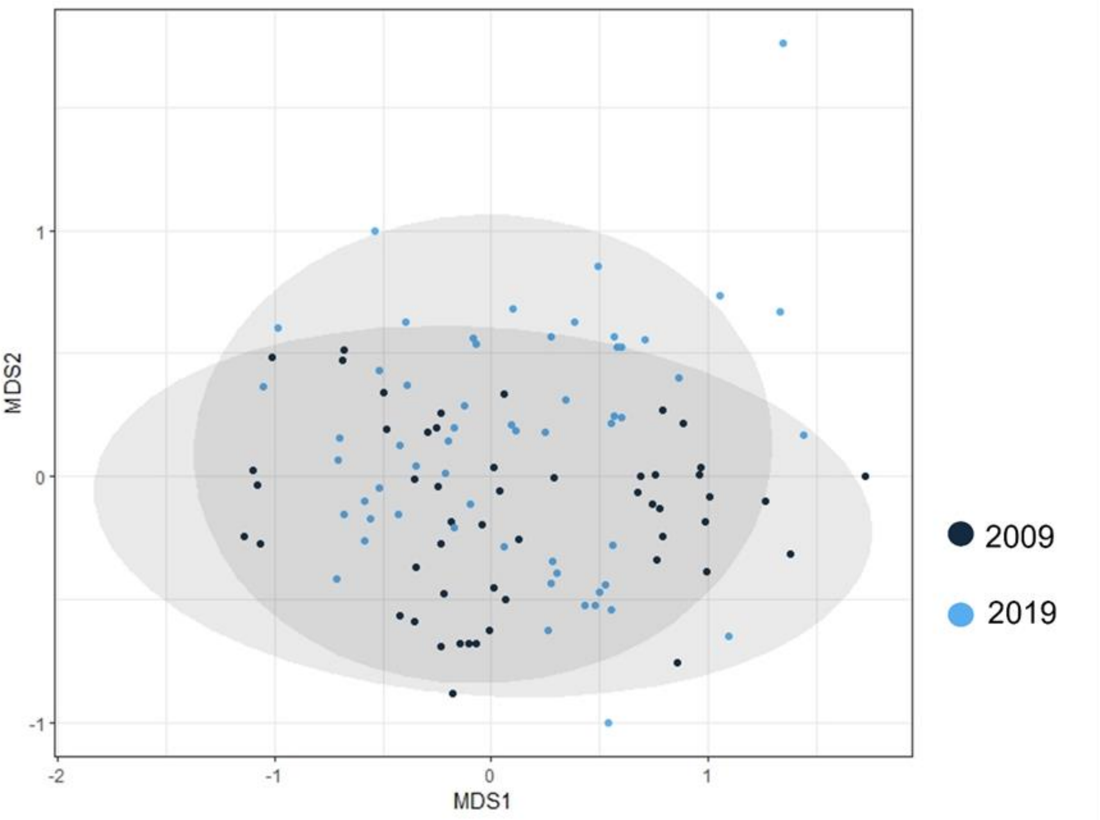


Figure 4.10 Temporal dietary variation in Temminck's red colobus of Cantanhez NP. Pairwise biplots from non-metric multidimensional scaling (NMDS) analysis were used to represent the significant variation in dietary composition of individuals in 2009 (dark blue dots, elipse) and in 2019 (light blue dots, circle). As samples from 2009 were only collected in the dry season (N = 60), only samples collected in the dry season of 2019 were used (N = 68).

In general, frequencies of occurrence in the 2019 dataset were higher than those in the 2009 dataset (Table 4.5). Five plants were common between the top 10 most frequently detected plants in the diet of western red colobus in 2009 and 2019. Although frequencies of occurrence differ for most plants, *Parinari excelsa* and *Leptoderris* spp. were detected in more than 35% of samples across both years. Four forest patches were sampled in both years: Cahgode, Cambeque, Jemberém and

Madina. Variation in the diet and dietary overlap specifically for these groups can be found in Table 4.4. Some forests show more distinct diets but this may be an effect of sample size.

Table 4.4 Dietary comparison of the same four groups of P. b. temminckii sampled with a ten year gap. Total number of plants detected in the diet, average plant taxa consumed per individual and the number of the same plant species detected in two points in time are described in the table.

Forest	*Sampling year*	*No. samples*	*Total no. plant taxa in diet*	*Av. no. plant taxa per sample*	*Overlaping plant taxa*	*Crop species*
Cahgode	*2009*	*9*	*17*	*5.2*	*8*	*0*
	2019	*18*	*16*	*4.7*		*0*
Cambeque	*2009*	*9*	*11*	*4.6*	*2*	*0*
	2019	*3*	*5*	*4.0*		*0*
Jemberém	*2009*	*22*	*15*	*3.7*	*9*	*18*
	2019	*9*	*14*	*5.8*		*7*
Madina	*2009*	*14*	*11*	*2.6*	*2*	*0*
	2019	*2*	*7*	*4.0*		*3*

In 2009, 5.6% of all food items were crops with a mean frequency of occurrence of 33.3%, in part due to the 18 dietary presence counts detected for mango across the 60 faecal samples. In 2019, crops were present in 20.6% of samples collected in the dry season, and they were detected across 10.8% of samples. Once again, mango was the most frequently detected cultivated item (13.2%).

Table 4.5 Temporal frequency of occurreence of top 10 plants in Temminck's red colobus diet. The ten most frequently detected plant taxa in the diet of *P. b. temminckii* in Cantanhez RNP at two points in time (2009 and 2019). **$F_o(\%)$**: Frequency of occurrence, the number of times plant taxa was detected across samples (2009, N = 60; 2019, N = 68).

P. b. temminckii, Cantanhez, 2009

Dietary taxon	$F_o(\%)$
Parinari excelsa	50.00
Cassia sieberiana	38.33
Leptoderris spp.	38.33
Mezoneuron spp.	33.33
Mangifera indica	30.00
Ficus spp.	28.33
Anisophyllea laurina	21.67
Milicia regia	18.33
Ceiba pentandra	15.00
Combretum spp.	13.33

P. b. temminckii, Cantanhez, 2019

Dietary taxon	$F_o(\%)$
Parinari excelsa	52.94
Leptoderris spp.	51.47
Ficus spp.	47.06
Combretum spp.	29.41
Treculia africana	29.41
Ceiba pentandra	23.53
Ixora spp.	23.53
Simicratea welwitschii	20.59
Trichilia prieureana	19.12
Cassia sieberiana	17.65

Significant differences in the mean number of plant taxa detected in red colobus faeces from Gola central and Gola south blocks were observed (W = 3563, *p*-value < 0.01), and variation in the dietary composition was also significant (LRT = 9.74, *p*-value = 0.001) but no plants contributed significantly for the variation. However, no samples in Gola south were collected during the dry season, which may have influenced the significance of the result. Furthermore, the intra-species variation probably associated with dietary preferences of different groups, may contribute to divergences in diet for the population (Fig. A.5.2 in Appendix 5).

4.5 Discussion

The results of this chapter demonstrate high species richness in the diet of western red colobus of Sierra Leone and Guinea-Bissau. For each subspecies, a small number of plants were the most frequently detected in faeces, hinting the importance of those taxa to *Piliocolobus* in each study site. Females and males did not have a significantly different diet, but variation was observed across seasons and time. Cultivated foods in the diet of *P. b. temminckii* were detected more often in samples from 2009 but more species were identified for individuals in 2019. Nevertheless, when compared with the diet of samples from the continuous and less anthropogenically disturbed forest of Gola, Cantanhez individuals consumed slightly more crops.

4.5.1 Dietary richness

Dietary richness of *P. badius* was in general higher than previous studies employing traditional methods to study the diet of red colobus across Africa for longer periods of time (Table A.4.1 in Appendix 4). This outcome was expected as initially proposed, and is probably a result of using a more robust technique featuring higher taxonomic resolution to detect rarer food items or more difficult to identify plants when using traditional methods (Pompanon et al. 2012; Sousa et al. 2019). In a comparison study examining the diet of red-headed wood pigeon, Ando et al. (2013) detected a significantly greater number of food plants using DNA metabarcoding (6.73 ±2.7) than microhistological analysis (1.42 ±0.6). Direct observations and morphological examination of plant parts in faecal samples has a reduction from the number of food items listed and those identified to the species or genus level. The population of *P. tephrosceles* in Kibale, Uganda is a good example of the problem researchers face when using traditional methodology: one group studied between 1972 and 1987 fed on a total between 91 and 167 plants, of which 85 were identified; for another group, which consumed 80 to 108 plants, only 70 species were identified (Struhsaker 2010).

A high diversity of plants was consumed by *P. b temminckii* in Cantanhez NP, but the population of *P. b. badius* in Gola RNP fed on almost 20 more plant taxa, exhibiting a much higher dietary diversity. The difference between the two study species of this project could reflect the poorer sampling in Cantanhez NP, but Hill-diversity indices suggest that the number of detections obtained for *P. b. temminckii* faecal samples covered a slightly higher percentage of the primate diet community than for the *P. b. badius* population of Gola RNP. Another possibility could be the overall species richness of the NPs vegetation, but the most recent botanical surveys registered similar flora numbers (Gola RNP = 899 spp.; Cantanhez NP = 863 spp.). However, species richness does not imply large quantities of food available, which is crucial for red colobus to meet their daily nutritional requirements as they feed on low-quality food items (Oates 1994; Struhsaker 2010). The result observed could indicate that some of the forest patches in Cantanhez NP do not have the same plant diversity found across Gola RNP.

Obvious intra-specific variation, commonly observed in the diet of primates (Wrangham 1977; Struhsaker 2010), could also explain the difference observed. Due to seasonal and annual variation in food availability, food items in the diet of red colobus may vary at the group and the species levels. Several years of data on the *P. b. tephrosceles* of Kibale forests (Uganda) suggest that the significant inter-annual variation in dietary composition reflects the complex diversity of the habitat (Struhsaker 2010). At the time of the study, Kibale was a relatively large old-growth rainforest, the type of habitat red colobus is thought to have adapted to. However, only a few plant species accounted for the majority of the species' diet, reinforcing the classification of the taxa as a 'specialist consumer' (Struhsaker 2010). The forest continuity of Gola RNP in contrast with the fragmented forest of Cantanhez NP may perhaps indicate that this is an important factor to consider in future feeding ecology studies and primate conservation plans. The importance of a continuous, well-preserved forest has recently been pointed out as critical in other aspects of red colobus conservation such as the maintenance of populations and its evolutionary potential (Minhos et al 2022, accepted).

Red colobus are known to feed on the largest and most common species. Unexpectedly, the only plant family that was both common in the forest and in red colobus diet was Fabaceae (subfamilies Caesalpinoideae and Mimosoideae). The other dominant Families in Gola RNP had low presence or were not detected in faecal samples (Euphorbiaceae and Annonaceae). The most frequently detected plant species, *Lophira alata* has not been recorded as a common plant in Gola RNP, but it was documented in associations with other dominant trees, such as *Heritiera utilis* and *Brachystegia leonensis* (Klop et al. 2008). *Lophira alata* was also an important food item for the population of Tai, Côte d'Ivoire (Wilkins 2017). Abundance data is currently being obtained in a survey carried out by a team of IBAP, so it is only possible to compare the plants detected in this study with the Families of the most common plants in the different types of forest in Cantanhez NP. These are likely to be Fabaceae, Chrysobalanaceae, Apocynaceae and Rhizophoraceae (Catarino and Palminha 2018), with the first two Families being also identified in this research.

4.5.2 Seasonal variation in western red colobus diet

Yearly fluctuation in the diet of western red colobus was observed across seasons in both NPs, but the results do not follow a specific pattern. Though the lack of phenology data during the time of the studies does not permit a direct correlation with young leaves, seed and fruit availability, previous studies conducted in the regions, indicate that these plant parts are more commonly produced in the dry season (Munro et al. 2013; Bessa 2014; Hockings et al. 2020). In Gola RNP, overall plant richness was higher in the rainy season than in the dry season, without significant differences in the mean average of plant taxa identified. The time of sampling in the rainy season in this study, is a period of low young leaf availability in the park. During these months, colobines usually feed more on mature leaves. Species richness results indicate that each individual does not increase the number of plant taxa it feeds on, but likely more groups are feeding on different plant species. Milton (1979) proposed that the protein-to-fibre ratio of food items may determine leaf choice in herbivores, and limits colobine populations size. It is also known that large groups have higher group feeding competition, and require more energy for travelling in search of clumped food (Wrangham et al. 1993; Chapman 2000). So, as a response to low preferred food availability in Gola RNP, red colobus may reduce group size and cover different parts of the forests in their daily travel distance, increasing the number of plant taxa in the overall diet.

In Cantanhez NP, more plants were detected in samples collected in the dry season, but the average number of taxa per sample was significantly higher in the rainy season. This result corroborates the maintenance of dietary diversity throughout the year, possibly since colobines do not feed on one specific plant part, as do frugivorous chimpanzees (Doran 1997), but rather a few plant species which can provide preferred food types (young leaves and seeds) and mature leaves in times of food shortage. It is evident that several years of data would be beneficial for a true representation of red colobus diet, especially in diverse and dynamic habitats where drastic habitat changes can occur, such as in Fathala Forest in Senegal which suffered a forest reduction of >50% in 30 years (Galat-Luong and Galat 2005), or Mbuzi Forest in Tanzania which has ferralitic soils, high elevations, and supports an agro-economic zone with intensified land-use due to high population growth (Kibaja 2014).

As pointed out in predictions 1. and 2., high species richness and seasonal variation in the dietary composition of western red colobus was evident, with a few plant taxa identified as most important for the species. This was clearer in Gola RNP with *P. b. badius* feeding on four main plants across seasons. These trees and shrubs can produce leaves mostly year-round, with simultaneous periods of flower and fruit production (Arbonnier 2002), providing food even at times of low food availability. *Nauclea* spp. was frequently detected in rainy season samples but not recorded at all in the dry season, most likely because flowering occurs in the beginning of the rainy season and evidence of *P. b. badius* feeding on young leaves and flowers of this genus have been recorded in Tiwai, Sierra Leone (Davies et al. 1999) and Tai, Côte d'Ivoire (Wilkins 2017). In Cantanhez NP, one plant, *Treculia africana*, was heavily consumed, with a detection rate of 97% in the rainy season samples. This tree is found in dense and riparian forests in the NP, a habitat preferred by red colobus. However, this plant was not identified as a common species in any of the forest types in the park. Neverthless, the tree appears to provide food annually as the flowering period is from October until February, and its large fruits, containing many seeds, can be present all year-round depending on region and environmental conditions (USDA 2015), supporting red colobus populations even in times of low preferred food availability. This result further supports the idea that red colobus populations are isolated in the small forest fragments of the park (Minhós et al. 2016). Four other plant taxa, including *Ficus* spp. and *Parinari excelsa*, featured strongly in the diet of *P. b temminckii* (F_o >30%) across seasons. Figs and fruits of *Parinari excelsa* trees were also important for *P. b. badius* in Tai and in Tiwai, and for *P. rufomitratus* in Kibale, Uganda (Davies et al. 1999; Ryan et al. 2013; Wilkins 2017). These two taxa are present in most habitats in the park, suggesting that red colobus inhabiting more degraded forest patches still have access to important foods. However, if deforestation continues these tree species which are now classified as Least Concern and are providing important food items to populations of the Endangered *P. badius* may decrease in density, putting primate populations at risk due to lack of food resources.

4.5.3 Differences in dietary composition across time

Dietary results for *P. b. temminckii* in the dry season at two points in time, 10 years apart, revealed no difference in the total number of plants detected in the faeces or the average number of plant taxa identified (as predicted in point 3). In a temporal analysis performed on the diet of *P. b. badius* in Tai forest (Côte d'Ivoire), where for the past 20 years transformations in the vegetation as a result of changes in the rainfall, fragmentation, and increased logging have taken place, there were clear reductions in dietary diversity and number of species contributing to the bulk with time (Wilkins 2017). These changes were not observed in this study, even when comparing the same forests sampled with a ten-year gap. The result likely supports the most recent information that Cantanhez NP forest did not suffer a reduction in vegetation cover since 1953 (Catarino and Palminha 2018). It appears that the food available 10 years ago may still support populations of red colobus in the park. In both years, *P. b. temminckii* fed frequently on *Parinari excelsa* (frequency of occurrence ~50% in each year), showing the importance of this tree for the population of Temminck's red colobus in Cantanhez NP. This tree is present in primary and secondary growth areas, savannas and palm groves, most of the habitat types present in the park, suggesting that groups of red colobus depend on few specific common plants widely distributed in the park, to meet their dietary requirements.

Even though a dietary overlap of 47.1% was detected across both years, more than half of the plant species were only consumed in 2009 or 2019. Thus, a significant variation in the diet of Temminck's red colobus was identified between the years. This result could reflect vegetation shifts taking place in the region, but to be precise, phenology or botanical surveys would have had to be conducted upon sample collection in 2009 and 2019. There is however, one other factor that could have an impact on diet variation across time. The forest where the highest number of red colobus were sampled, Catombói, was not sampled in 2009. The population in this forest, in comparison to four other forests sampled in both years (Table 4.4), shows higher plant diversity in the diet (N = 52, no. taxa = 29, average no. taxa per sample = 6.1). This values also indicate that Catombói forest could be a healthier forest than other fragments in the park, supporting a large population of Temminck's red colobus, which should be further investigated for a more focused conservation plan, and to determine why this forest is more appealing to this primate than other areas of the park.

4.5.4 Crop consumption in a specialist diet

Notably, cultivated food items had a high presence in the dietary composition of both time points in Cantanhez NP, than the population of Gola RNP, supporting prediction

4. The inclusion of crops in the diet of a highly forest dependent primate is probably a response to living in a more degraded habitat and nearer cultivated fields. This behaviour has been observed in other populations of red colobus that could not cannot meet their dietary requirements from preferred wild food due to forest degradation (Galat-Luong and Galat 2005; Kibaja 2014). The crops detected in the diet of red colobus in Cantanhez NP are usually present near settlements, in peoples' gardens or are cultivated as monocultures in orchards. Some exemplars of mango trees may are located in the dense forest, but in general the results of this study demonstrate that *P. b. temminckii* is foraging on grounds that are commonly used by humans. Crop consumption was higher in the diet of red colobus representing the dry season of 2019, but the total frequency of occurrence was lower than in 2009. Mango consumption during the trees fruiting season (dry) was the reason for this result. This species is common in the region and it was present in 30% of samples from 2009 and 13.2% from 2019. However, although in the most recent samples detection of the crop was spread across two forests, in 2009 all mango detections were found in individuals sampled in Jemberém forest close to the settlement, where a group of red colobus is known to forage and large mango trees are present. One result that was unexpected, was the presence of crops in the red colobus group from Catombói, a forest suggested to provide better wild food resources to the species (section above). Perhaps some of the farm fields in this forest extend further away from the village, providing a more secure area to forage without being exposed to humans. One point that may have some influence, is the fact that one of the collaborators in this study is from the village near this forest and because he brings awareness to the rest of the community about primate conservation, people's behaviour towards primates might be less hostile. This may in turn allow red colobus to feel less threatened and confident in exploring easily accessible high-quality food items.

Although in Gola crops were also present in *P. b. badius* diet, they had a lower frequency of occurrence, and the most frequent crop was Coffea spp. This tree is cultivated in orchards that are managed under an agroforestry system. As most species are intolerant to direct sunlight and benefit from a soil enriched by the fallen leaves of high canopy trees (Alemu 2015), farmers in Gola region have planted coffee trees under native tall trees (information provided by conversations with farmers during fieldwork). In fact, another crop that was detected in the diet of red colobus in Gola RNP, is *Enterolobium* spp. which is used as a shading tree in coffee plantations of South America (Cortés-Delgado and Sosa 2014). This strategy improves the production of coffee but it appears to provide a suitable habitat for the Endangered Bay colobus. This may be a good conservation tactic, but the impact of hunting needs to be assessed to evaluate the sustainability of the practice.

4.5.5 Contribution to primate conservation

Degradation of habitat quality due to vegetation loss, fragmentation and transformation to farmland is one of the major threats to population sustainability (Struhsaker 2010; Linder et al. 2021). One solution to minimise biodiversity loss is to implement new and/or improve management of existing protected areas. However, not all protected areas are suitable habitat for primates (e.g. sand dune vegetation, high altitude forests) (Estrada 2006) and, although important for wildlife and ecosystem conservation, many do not offer the best conditions for primate populations to thrive (Chape et al. 2005). Many are poorly managed, understaffed or simply exist on paper, and are commonly small vegetation islands surrounded by altered landscape (DeFries et al. 2005; UNEP-WCMC, & IUCN 2022). In this study, we conclude that Gola RNP is possibly providing enough natural resources for western red colobus to maintain their dietary requirements but evidence of some level of dietary adaption to habitats where humans are present is starting to be evident, as some individuals included crops in their diet. However, in the case of *Coffea* spp. crop, it may be a sustainable system that allows for wildlife to co-use the same space as people use for economically important plants. Future research would be essential to understand better how primates use these agroforest areas, and plan along with local communities, the most sustainable procedures in this and other plantations. Some work is already being developed in Gola RNP with cocoa plantations which could serve as a starting point to explore the growth of other crops along with endemic species.

In Cantanhez, the complex social-ecological system may be having a significant impact on Temminck’s red colobus as their flexibility to a disturbed environment is clearly visible from the data obtained in this research. Despite showing a flexibility behaviour that currently allows the species to cope with alteration in wild food resources, habitat reduction or replacement by monocultures, which does not favour biodiversity, is leading this primate to live in smaller forest fragments with poorer quality and fewer quantity of natural foods available. Even though dietary species richness of red colobus in 2009 does not differ significantly from the diet of the current population, a more detailed research including phenology data needs to be conducted to understand if variations in the diet composition observed over time are indicative of a decline in the abundance of some important plants to Temminck’s red colobus. The identification of plant species contributing largely to the species diet should be considered for future reforestation campaigns. Strategic plant species and locations must be well defined for the sustainability of the project, including plant growth success and feasibility of habitat use by red colobus and other species.

Anthropogenic pressure will continue to be exerted on natural habitats and weakly protected areas due to a lack of stable economic growth in many rural tropical regions (FAO 2020). Deforestation of the natural habitat of most primate species, has severe consequences in the long term to most species, resulting in population decrease and, in some cases, extinction. Therefore, it is important to use integrative approaches to understand how primates respond to habitat transformations in space and time, to provide and implement sustainable and achievable conservation strategies within and outside protected areas.

CHAPTER 5. Wild plant overlap between sympatric humans and non-human primates in a continuous rainforest and an anthropogenic landscape

Cocoa empty shells drying under the sun for further use by villagers in Gola Rainforest National Park.

5.1 Abstract

Most environments inhabited by non-human primates either have humans present or are anthropogenically modified. In these anthropogenic landscapes, sympatric humans and non-human primates can share natural resources. Understanding the complex social-ecological dynamics of such systems not only contributes to the conservation of threatened primate species, but also plants that have an important place in people's livelihoods. In this research, we applied a mixed-methods approach to estimate the level of wild plant overlap between rural human communities and non-human primate populations living in two national parks of West Africa. Plants consumed by western red colobus (*Piliocolobus badius*) and western chimpanzees (*Pan troglodytes verus*) were identified using high-throughput molecular biology sequencing. Simultaneously, records of the most important plants for humans were obtained by interview. Results showed some degree of resource overlap at both sites, with higher rates at the more disturbed and fragmented forest of Cantanhez, Guinea-Bissau, and between humans and red colobus. Dietary richness and niche analysis when comparing the continuous forest of Gola, Sierra Leone, and Cantanhez, suggests dietary flexibility in both non-human primate species. Although red colobus appear to be better adapted to disturbed habitat than chimpanzees, high within species variation in dietary composition may have affected the results. Notwithstanding, a higher number of cultivated items were detected in the diet of red colobus, though chimpanzees showed a higher frequency of occurrence for the fewer crops that were found to be ingested. Nevertheless, some key plants were found to be important for both humans and one or both non-human primates across sites (*Ficus* spp), and within national parks (e.g. *Carapa procera* in Gola, and *Parinari excelsa* in Cantanhez). Additional multi-site and cross-species research needs to be conducted and extended to evaluate the degree of overlap between sympatric species and disentangle the complexity of such systems. However, investigations such as the one presented here, can inform natural resource management facilitators to guide, together with local communities, habitat restoration programs to deter further deforestation and

contribute to non-human primate conservation, and integrating traditional sustainable land management practices.

5.2 Introduction

5.2.1 Background to study

One of the main threats to non-human primates (here after primates) is habitat loss and degradation, resulting from human population growth and associated transformation of the natural habitat, such as land conversion to agricultural fields or urban areas (Estrada et al. 2012; McKinney 2015). Most primates left residing in degraded habitats will see their food resources diminish, sleeping or hiding places disappear, chances of surviving natural disasters reduce, and exposure to pathogens or predation increase (Schwitzer et al. 2011). However, the biological and ecological diversity that characterises this group of animals, provides them with the capacity to respond to habitat alterations in many ways. Some primates are more resilient and can sustain major shifts in the surrounding environment, such as Cercopithecine monkeys that are among the most conspicuous primates living in heavily disturbed regions (Albert et al. 2014). As diet is an essential factor for species survival, even the most vulnerable appear to adopt strategies that allow them to cope with modification and degradation of their food resources (Nowak and Lee 2013). In most landscapes occupied by primates, humans exert a heavy presence and use the habitat resources, including wild plants (Bharucha and Pretty 2015). Usually, human populations sharing habitat with primates live in extreme poverty and depend on natural resources for their daily requirements, mainly nutrition and traditional medicine (Heubach et al. 2011; Angelsen et al. 2014). Hence, in contemporary primate conservation projects, people’s needs and use of the forest should be considered. Ultimately, primates are key elements in ecosystem sustainability, for example as important seed dispersers for the maintenance of old-growth forest and the development of regenerating forest (Chapman and Dunham 2018), contributing to human well-being. Variation in habitat use by humans, and primate responses to habitat disturbance is very much case specific, pointing to the importance of taking an integrative approach to address this complex social-ecological system. Ethnoprimatology, the science that studies the human-primate interface, uses interdisciplinary methods and perspectives to explore the social and ecological interactions between humans and other primates (Fuentes and Hockings 2010; Fuentes 2012; McKinney and Dore 2018). As both humans and primates depend on the forest, primate conservation should be prioritised in regions of fast habitat transformation and population growth.

5.2.2 Sympatric human and non-human primates

Humans and other primates shared ecosystems for millennia, sometimes in a holistic and beneficial way, and other times in more negative and conflicted manner (Tutin and Oslisly 1995). Sympatric organisms modify their own niches as well as those of other species, through their activities and choices (Odling-Smee et al. 2013). This is known as “niche construction” and it can be observed in many areas of the world where humans and primates live in close proximity, overlapping spatially and ecologically. Farmers, hunters, gatherers, fishers and foragers not only extract naturally occurring resources, but they also manage and amend them to increase the productivity and stability of useful plants and animals (Bharucha and Pretty 2015). Ideally, this natural resource exploitation would be sustainable if the plant parts collected were not critical for the long-term survival of the species, and if intensity and frequency of harvesting was low (Belcher and Schreckenberg 2007; Hernández-Barrios et al. 2015). In times of environmental and economic uncertainty, non-timber forest products (NTFPs) provide food security for an estimated 880 million people worldwide (FAO 2020), but are also highly valued in traditional medicine and culture (Bharucha and Pretty 2010). For primates, food availability influences growth, group size, social interactions, and many other biological and behavioural characteristics (Wich et al. 2006; Marshall and Wrangham 2007). For species that are specialised in certain food resources, habitat quality and food availability is vital for survival. Chimpanzees do not have a specialised gut and so should rely more on high-quality food, such as ripe fruits (Temerin and Cant 1983; Wrangham et al. 1998). To meet their food requirements when seasonal ripe fruit availability decreases, chimpanzees travel further and for longer (Wrangham 1977). Red colobus on the other hand, have a very specialised gut morphology allowing them to digest low-quality food. Plant parts comprising leaves, both young and mature, seeds, and unripe fruit from a wide diversity of plant species are consumed by all colobines (Maisels et al. 1994; Oates and Oates 1994). However, these primates rely only on a reduced number of species which account for a large proportion of the annual and total diet (Struhsaker 2010; Wilkins 2017), characteristic of resource specialists.

The niche theory ‘resource diversity hypothesis’ suggests that the niche of co-occurring consumers can diverge or expand as an effect of the diversity of resources available (Costa-Pereira et al. 2019). In natural environments, overlap of food resources among red colobus and chimpanzees might exist and could result in interspecific food competition (Conklin-Brittain et al. 1998; Wrangham et al. 1998). However, data on consumption of common plant resources by the two primates is limited, with research focusing mostly on predator-prey interactions (Boesch and Boesch 1989; Bshary and Noë 1997; Uehara 1997; Kane and McGraw 2018). Another dimension of the investigation presented in this chapter, which has received little attention by researchers, is the degree to which humans and non-habituated primates co-utilise wild plants. Even though research on primate interactions with people has increased since 2000, most studies have focused on dietary adjustments of chimpanzees and macaques in anthropogenic habitats (see McLennan et al. 2017).

5.2.3 Dietary flexibility in degraded habitats

Landscape degradation primarily affects the diversity, density, distribution, and nutritional content of plant items, and in turn has direct impacts on primate survival. When changes in food availability take place in altered habitats, primate density, dispersal range, reproduction success and social composition may be affected (Johns and Skorupa 1987; Schwitzer *et al.* 2011; Hohmann *et al.* 2012; Almeida-Rocha *et al.* 2017). Nonetheless, the form of disturbance may be crucially important for species survival along with its ecological features. In moderately disturbed forests, Johns and

Skorupa's (1987) review identified that 44% of the variation in species response was determined by body size and degree of frugivory. Smaller species survived better in disturbed habitats, and the percentage of frugivory correlated negatively with survival ability. For food specialists red colobus and chimpanzees, sensitivity to alterations in the forest composition should be high, but some research suggests that they can be flexible in their diet. Chimpanzees in Bossou, Guinea, did not actively avoid foraging close to roads and paths, and they showed the same selection ratio for coffee plantations as mature forest during the wet season (Bryson-Morrison et al. 2016). In Kibale NP, Uganda, home to several primates, variable vegetation occurs which reflects the intensity of commercial logging that took place before 1994. Outside the protected area, the scenario is no better, with small patches of unprotected forest often disturbed by human activities. Nevertheless, red colobus monkeys are one of the 13 primate species in this site that can live throughout the different habitats and interact with the human population in crop-feeding events (Milich 2017). Despite their dietary flexibility, it is still unknown if these animals can survive in such conditions for a long period. The likelihood is low, as primate population trends are decreasing, mostly as a result of habitat loss, and these animals depend on trees for many reasons other than food security, such as protection, space for social interactions, and sleeping sites (IUCN SSC 2020; Linder et al. 2021). Chimpanzees appear to cope better with habitat changes than red colobus, mainly due to their flexible and adaptive behaviours in response to perceived risks in anthropogenic landscapes (Hockings et al. 15). In Bossou, Guinea, chimpanzees travelled and rested more in forested habitat, but used highly disturbed habitat types for socialising (Bryson-Morrison et al. 2017). Nevertheless, this population regularly visits cultivated fields to forage on crops, increasing group cohesiveness and vigilance behaviour, and cross roads daily to move through forest and farms, a risky strategy given human presence and vehicle traffic (Hockings 2007).

Predicting species sensitivity to habitat loss and fragmentation is important for conservation conducted in human-dominated landscapes. Niche is a foundational concept in ecology which helps to predict biodiversity patterns and species co-existence (Chesson 2000). In habitats where resource availability is limited, species' natural niche and competitive interactions may change, although seasonal and annual variation is a constant in species diet. Different dietary requirements may influence an animal's response to disturbed environments, and patterns in resource use may indicate human-induced activity shifts (Swihart et al. 2003; Smith et al. 2018). An intuitive idea is that when diversity of available resources is smaller, co-existing species' niche breadth would be narrower and overlap increases (Colwell and Futuyma 1971; Pianka 1973), but empirical support for niche alterations in multiple dimensions is lacking (Costa-Pereira et al. 2019). However, unexpected behavioural responses can be traced to characteristics of the changing landscape, such as selectively increase the consumption of a particular food source resultant from the disturbance, over an important food item (Johns and Skorupa 1987). When exposed to anthropogenic modifications to their natural food, *P. badius* and *P. t. verus* adopt different strategies to overcome food shortage. For example, red colobus increase the consumption of plants that are more commonly available in the disturbed habitat in their diet (Kibaja 2014; Wilkins 2017), while *P. troglodytes* groups travel between remaining forest fragments and feed on crop plantations (Naughton-Treves et al. 1998; Onderdonk and Chapman 2000). In anthropogenic environments, crops appeal to chimpanzees due to their high digestibility and low toxicity, but also provide greater foraging efficiency over natural foods (Robinson 1986; Strum 1994). While crop feeding has been documented in colobines , it is usually observed during a period of low availability of alternative food resources (Siex and Struhsaker 1999; Kibaja 2014), while the same association has not yet been observed in chimpanzees (Hockings 2007), suggesting higher degree of dietary flexibility in altered environments.

5.2.4 Integrating disciplines to study primates in anthropogenic habitats

Modern primatology was arguably initiated in the 1960s and it was mainly directed at small populations of habituated primates living in natural landscapes, but due to the growing world population and subsequent land transformation, it is imperative and necessary that researchers study species at anthropogenically impacted sites (Hockings et al. 2015). It was only after the 2000s that an increasing number of publications included research on primate behaviour and interactions with people, in anthropogenic habitats (McLennan et al. 2017). Effective species conservation can only be achieved with a deep understanding of the species and its ecological threats, as well as the social and governance context of those threats (Whitty 2018). Ethnoprimatology is a field of research that integrates different disciplines, using a mixed-methods toolkit (qualitative and quantitative methods), to assess the human/non-human primate interface (Fuentes 2012; Dore et al. 2017; Setchell et al. 2017). The benefits of applying this interdisciplinary approach is relevant for behavioural ecology, cognition and evolution of primates, but also applied primate conservation (Fuentes and Hockings 2010; Lee 2010). In general, primate feeding ecology studies focus on food acquisition and processing, habitat utilisation, foraging strategies, ecology and sociality (Robbins and Hohmann 2006), but usually exclude any interaction with human foods (e.g. crops) or wild plants exploited by humans. Similarly, in Ethnoprimatology, a wide range of topics are covered, such as people's perceptions of primates and their behavioural flexibility to disturbed environments, but not many focus on the overlap of resources between humans and primates, though the incorporation of human food in primate diets is one of the most commonly documented adjustments of the taxon (McLennan et al. 2017). The reason why so few investigations have been conducted in the co-use of wild plants in anthropogenic habitats may be due to a number of factors. Primarily, most researchers working in this field have a biological sciences background with little or no knowledge in social methods and analysis (Newing 2011). The need to use different methodologies from distinct disciplines (e.g. anthropology and primatology), collect social and biological data within the same time frame, and have good botanical knowledge to collect correct taxonomic information on a wide range of plants used by humans and primates may discourage most scientist to use a cross-disciplinary approach (Hockings et al. 2020).

Of the few studies which examined the co-use of plants by humans and primates, most focus on one plant species only, which is important for both groups (Kinnaird 1992; Reynolds et al. 2012; Sheil et al. 2012; Ramon 2017). Even though this approach can produce important information on the spatial and temporal overlap of a source that is commonly exploited by both, it does not reflect the real use of wild resources across species, seasons, and space. A better methodology is to concentrate on a specific type of plant that is heavily consumed by a focal primate species but also important for sympatric humans, as is the case of fruit for chimpanzees (Hockings et al. 2020). Two studies that looked at a broader use of wild plants by humans and primates, observed a high overlap of wild resource use. In Bossou, Guinea, people utilised far more wild plants for many different purposes than chimpanzees fed on, but they commonly used around 86 species (Sugiyama and Koman 1992). In Lore Lindu NP, Sulawesi (Indonesia), the top five plants that constituted 80% of the tonkean macaque diet were among the top ten most salient resources for villagers (Riley 2007). Many of the interactions between sympatric humans and primates reported in Ethnoprimatology research, focus on negative rather than positive or neutral experiences. In some cases, humans can extensively harvest an important plant in primate diet, and most primate crop-feeding events are seen as damaging to the livelihoods of rural communities. But collectively, publications conducted in shared environments and reviewed in McLennan et al. (2017) show that primate behaviour is not always detrimental to humans. Forest-dwelling primates in agro-ecosystems act as potential pollinators (Estrada 2006), and seed dispersers (Chapman and Dunham 2018), they can also contribute to forest regeneration (Albert et al. 2014), and create seedling growth hotspots through excrement deposition (Kalbitzer et al. 2019). Primates contribute to the continued forest dynamic and existence (Chapman and Onderdonk 1998), in mature and disturbed habitats. For example, even though red colobus are considered seed predators, digesting most of the seed, rather than seed dispersers, the population of *Piliocolobus tephrosceles* in Kibale, Uganda did not limit the density of seedlings of some of their preferred foods. Instead, seeds and/or unripe fruit from the most often consumed plants (e.g. *Celtis* *durandii*, *Funtumia latifolia*, *Mimusops bagshawei*) had greater seedling and sapling population densities than those not eaten by colobines (Struhsaker 2010). In Bossou, Guinea, cacao seeds spat by chimpanzees or ingested whole and later deposited through faeces in plantations, successfully grew to produce fruit as a result of farmers' maintenance of the area (Hockings et al. 2017).

5.2.5 Aims and hypothesis

The overall aim of this chapter is to understand how much wild plant overlap exists between sympatric primates and humans in an agroforestry landscape in comparison to a continuous and less anthropogenic landscape. Comparisons of the dietary richness and composition, as well as crop consumption of two primates with different food requirements will be conducted in two different locations. This information will allow for an evaluation of the species' flexibility to a fragmented habitat and their importance as seed dispersers and the quantification of the co-use of wild plant resources between local human populations and primates.

Three specific goals developed for this research are to:

- (a) perform an inter-species comparison of the diet of the specialist western red colobus and the generalist western chimpanzee, in the continuous and diverse Gola RNP and the fragmented landscape of Cantanhez NP;
- (b) assess primate dietary flexibility to a disturbed landscape by comparing differences in the diet of the two sympatric primates in the continuous and fragmented forests of each NP;
- (c) estimate the co-utilisation of wild plants by local human communities and the two primates in the two distinct study sites, and identify important plants that could be considered in habitat regeneration conservation programmes.

Predictions:

1. Chimpanzees will reveal a narrower dietary niche breadth and lower species richness than red colobus, as the species is a ripe fruit specialist. This

difference will be less evident in the agroforestry habitat of Cantanhez, as a result of reduced preferred food quantity;

2. Plant species richness will be higher and niche breadth wider in Gola RNP, where food availability is less restricted, but niche overlap between red colobus and chimpanzees will be more pronounced in Cantanhez NP due to reduced variability of available food species;
3. Both primates will consume fewer or no crops in the continuous habitat than in the more anthropogenic landscape of Cantanhez, but chimpanzees will have a higher incidence of cultivated plants in their diet as they are more dependent on ripe fruit availability, while red colobus can overcome food shortage with other food items, such as mature leaves.
4. Plant overlap in Cantanhez NP will be larger due to the nature of the habitat occupied by humans and the co-existing red colobus and chimpanzees. This co-use will be observed in both wild plants and crops, though chimpanzees will probably share more plants with humans because both prefer fruits.

5.3 Methods

The anthropological methodology used in Chapter 2 was integrated with the molecular biology techniques presented in Chapters 3 and 4 to study the co-use of forest resources by two non-human primates and humans. One field work period of five months was conducted at the study site of Gola Rainforest National Park (GolaRNP) in Sierra Leone, and the second period of data collection took place in Cantanhez Forest National Park (Cantanhez FNP) in Guinea-Bissau. These two West African protected areas experiencing different levels of human pressure, are home to populations of the threatened western red colobus (*Piliocolobus badius*) and the critically endangered western chimpanzee (*Pan troglodytes verus*). Moreover, several human communities also live in the region and use the forest and its resources daily. Non-invasive samples from red colobus and chimpanzees were collected at known sleeping and feeding sites, and other areas used by these primates, and oral interviews humans were conducted in villages near those same sites. DNA extraction and high-throughput plant DNA sequencing was carried out in the Molecular Ecology laboratory and the Genome Hub at the School of Biosciences, Cardiff University. Information from the interviews was coded to ensure anonymity and analysed for demographic purposes and to evaluate the use of wild plants by people in each NP. Ultimately, all data will be used to answer questions on the use of wild plants by humans and non-human primates in protected areas with different anthropogenic stresses.

5.3.1 Study sites

5.3.1.1 *Gola Rainforest National Park*

Gola RNP was visited in April-May (dry season) and June, October-November (rainy season) of 2018, situated in the southeast of Sierra Leone. The protected area of ~750 km^2 tropical rainforest was established as a National Park in 2011. The dense lowland moist evergreen and semi-deciduous forest has an almost continuous vegetation cover and a clear demarcated boundary (Lindsell and Klop 2013). The park is heavily managed for forest conservation by park rangers, community research assistants, and national and international researchers. The forest is dominated by trees reaching 40m in height in the primary forest and features many species of birds, butterflies, fish and mammals, including the iconic pygmy hippopotamus, the forest elephant and nine species of non-human primates (Klop et al. 2008). The 122 forest edge communities of mainly Mende people, are situated on the outskirts of the protected area with a population of ~23,500 inhabitants (Bulte et al. 2013). They are allowed to use the forests resources in a sustainable manner, and although hunting, logging and mining are forbidden, some episodes still occur putting at risk populations of threatened plants and animals. A detailed description of Gola RNP can be read in Chapter 1, section 1.7.1.

5.3.1.2 *Cantanhez Forest National Park*

The protected area of Cantanhez in the south of Guinea-Bissau was gazetted in 2008 to preserve one of the last subhumid forests in the country. The region covers about 1,000 km^2, with 16 patches of dense forest surrounded by savanna, mangrove and agricultural fields (Catarino and Palminha 2018). High floral and faunal biodiversity are observed, with many species of fish, reptiles and mammals, including seven non-human primate species (IBAP 2007; Vieira et al. 2019). Further details on the park and a table comparing both study sites are available in Chapter 1, section 1.7.2. During March-April of 2019, non-human primate faecal samples were collected and interviews with people from 15 villages were conducted. Later in the year (November-December), further non-invasive sampling took place to cover the diet of primates in the rainy season. A total of 110 villages with 24,000 people from several community groups live inside Cantanhez FNP. The majority are small scale farmers who conduct other activities which involve collecting and/or using forest products (e.g. carpenters, palm oil producers). Activities such as deforestation and hunting are not permitted in the park, but limited formal protection and weak implementation of regulations threatens the biodiversity of the region (Hockings and Sousa 2013). Contrary to what would be expected in anthropogenic habitats, vegetation cover has increased from 1953 to 2003. However, this is mainly due to an increase of monoculture of cashew trees (*Annacardium occidentale*), more prominent in the north of the park (Catarino and Palminha 2018) where red colobus are difficult to observe (Minhós 2012, and pers. obs.).

5.3.2 Study species

In this section, we focus on population densities of the study species in each NP and their general diet. For information about the species and specific populations, please refer to Chapter 3, section 3.3.2 for the western chimpanzees, and Chapter 4, section 4.3.2 for the western red colobus.

5.3.2.1 *Red colobus, Piliocolobus badius*

The western red colobus is the only red colobus that subdivides into two subspecies: the Bay colobus (*P. b. badius*) and Temminck's red colobus (*P. b. temminckii*). In 2008, roughly 14,800 Bay colobus were thought to inhabit the continuous protected forest of Gola. More recent information suggests a smaller number (>5,000), though updated densities are yet to be estimated for one of the most viable populations. However, trends from recent studies in Tai NP (Côte d'Ivoire) suggest a decline (Oliveira 2017). In the fragmented and anthropogenically altered habitat of Cantanhez, an important and relatively large population of *c.* 300 adults occupying mostly the south of the park, is threatened mainly due to deforestation and hunting (Minhós et al. 2013; Minhós et al. 2016).

Colobines have a distinct gastrointestinal morphology, allowing them to feed on items with lower nutritional quality and more antifeedants (natural chemicals used as a plant defending strategy) (Fleagle 2013). For some time, dietary research identified this group of animals as folivores. However, fruit has been concluded as the most important item for populations of *P. b. temminckii* in Abuko (Gambia) (Starin 1991; Oates and Oates 1994) and for individuals in Fathala (Senegal), along with seeds (Gatinot 1977; Gatinot 1978). Further, if food item consumption scores, gathered in an intertaxa analyses performed by Struhsaker (2010), are added, it may be more accurate to say that red colobus are leaf and seed eaters, often with the inclusion of unripe fruit. Occasionally, invertebrates, twigs, bark, and soil are also ingested (Struhsaker 2010; McGrew 2014). Various studies of red colobus feeding ecology recorded an average of 50 plant species (min = 17, max. = 89) in their diet with the most important plants differing between studies (Table A.4.2 in Appendix 4). The number of plants in the diet of these populations of red colobus are only a proportion of the plant species available in their habitat. Some red colobus populations living primarily in degraded habitats have been observed to complement their diet with cultivated food items. Most studies of western red colobus have been carried out in Senegal, The Gambia and Côte d'Ivoire, with only one study in Sierra Leone, on

Tiwai Island (Davies et al. 1999). This patch of forest is located at the southwest tip of Gola RNP and the study examined patterns of frugivory in *Colobus polykomos*, *Pilicolobus badius badius* and *Procolobus verus*. To date, no dietary studies have been developed for any of the *P. b. temminckii* populations of Guinea-Bissau.

5.3.2.2 *Western chimpanzees, Pan troglodyte verus*

Chimpanzees are the most common of all African apes, occupying several habitat types from dense rainforest to savannahs and farmlands, across the African forest belt (Butynski 2003; Oates and Nash 2011). The Critically Endangered western chimpanzee (*P. t. verus*) is estimated to have suffered a major decline of 80% between 1990 and 2014, with a reduction in its range of 20% (Klailova et al. 2018). Currently, this subspecies inhabits eight West African countries with populations in Gola RNP (Sierra Leone) and Cantanhez NP (Guinea-Bissau). The continuous and relatively remote Gola forest experiences low levels of deforestation and other human induced activities. These characteristics may provide a good habitat for chimpanzees to persist (Heinicke et al. 2019). However, the latest density survey, estimated a population of only 300 individuals spread across nearly 750 km^2 of evergreen rainforest. This number is lower when compared with the estimated 376-2,632 individuals living in the fragmented forest of Cantanhez NP (Torres et al. 2010). Both populations are threatened mainly due to poaching (either for illegal killing or live capture for pet trade) and by deforestation in Cantanhez (IUCN SSC 2020).

Chimpanzee diet consists mainly of plant matter but these animals are considered frugivorous, as the most common food item is ripe fruit (Tutin et al. 1997; Morgan and Sanz 2006; Pruetz 2006). Chimpanzee diet varies greatly and, similarly to red colobus, shifts are observed from month to month (Boesch et al. 2006). Direct observation of chimpanzee feeding ecology has revealed several plant parts from wild species (e.g. seeds, flowers, bark, and gum), honey, invertebrates and sometimes vertebrates such as monkeys (Yamagiwa and Basabose 2006; Hockings et al. 2009; McGrew 2014; Bessa et al. 2015a). Tool use has been recorded for nearly all chimpanzee populations and they use tools in food consumption (e.g. stones to crack nuts, thin leaves and twigs to extract termites) (Boesch and Boesch-Achermann 2000; Sanz et al. 2009). Crop consumption in African chimpanzees is common, with 36 cultivars being detected in a country-wide study (Hockings and McLennan 2012). In Sierra Leone, only one study was conducted on the diet of chimpanzees. Jones and Cave (1960) identified fruit and leaves from the original mature forest as the main bulk of the diet, but also identified other food items originating from dense secondary forest ("farm bush"): two types of invertebrates and three crops. In Guinea-Bissau, researchers have studied the diet of chimpanzees, but one specific population in Cantanhez FNP, has been studied in depth due to their potential dietary overlap with human communities. The two investigations that evaluated wild plant as well as crop consumption were both conducted in 2015 (Bessa et al. 2015b; Carvalho et al. 2015).

5.3.2.3 *Human population in Gola RNP*

The interview sample in this study reflects the characteristic human demography of the region. Respondents were mostly between 15 and 64 years (67.3%), more men were interviewed (63.5%), and almost all had Islamic beliefs (>90%). Official education attendance was low (7.7%) probably because most forest edge community villages (FEC) are distant from schools and even basic education is expensive. Farming is the dominant occupation in the region, and despite having crop plantations as a source of income, none were large scale farmers. Cocoa and coffee plantations were more common, but pineapple, banana, kola nut and rice are grown by people living in FECs. Because interviews were aimed at targeting the entire community composition, only two healers were interviewed. Yet, the majority confirmed using wild plants collected in the protected area of Gola RNP (67.3%) for several purposes: traditional medicine, construction, food, art craft and others. Perceptions on plant use restrictions (e.g. law enforcement) and plant use variation between the central and south regions of the park are discussed in Chapter 2, section 3.3.1 and 3.4.

From the respondents who claimed to use wild plants, 70% used it in traditional medicine. This most likely results from the poor healthcare services experienced particularly in rural areas, where people have to travel about half a day to reach a clinic (Bulte et al. 2013; Robinson 2019). It is typical for Sierra Leoneans to travel a few kilometres to the forest and harvest plant resources that can be used for initial treatments (Robinson 2019). In Gola RNP, it is mostly men who travel to the dense forest to collect wild plants for themselves or other people, as there are risks associated with this activity, such as encountering wild animals (Chapter 2, section 3.3.1). A small number of respondents commercialised this activity (11.5%), but most used it only for domestic purposes. A total of 49 plant items were recorded across 52 interviews, and 44 were identified to the species. Gola forest is dominated by Euphorbiaceae and Fabaceae species, and the plants most utilised by people belonged to Families Fabaceae, Rubiaceae and Arecaceae. Respondents used on average five wild plants which are collected all year round as necessary, regardless of the distance from the village to the forest boundary. Nevertheless, many respondents also used plants growing in the community forest (vegetation around the villages that resembles the composition of the mature forest) to limit long distance travel. On some occasions, people travelled to the forest to collect species that can only be found in the forest, such as rattan (*Eremospatha macrocarpa* or *Laccosperma secundiflorum*). The most important plants for people living in FECs around Gola were bitter kola (*Garcinia kola*), bush ataya (*Massularia acuminata*), rattan (*Eremospatha* spp.), west African black pepper (*Piper guineense*) and west African laburnum (*Cassia sieberiana*). A list of frequency of mentioned plants can be found in Appendix 2 (Table A.2.3). All top plants are used in traditional medicine (Jusu and Sanchez 2013; Munro et al. 2013) except *Eremospatha* spp., which is used in construction and furniture.

5.3.2.4 *Human population in Cantanhez NP*

The 56 interviews conducted in Cantanhez NP revealed demographic data resembling those obtained in national surveys and other studies in the region. Most respondents were elders (41.1%), with more than one third being women (36.6%). People representing eight ethnic communities were interviewed, reflecting the large diversity of community groups in the region (Temudo 2009; INE 2017). Similarly, four different religions were also covered by the interviews, but nearly 80% of the people identified as Muslim. A high number of respondents (69.6%) had some education, either official or Arabic with more than one third reaching secondary school. Seven healers were interviewed by chance, but their main occupation was farming, as with nearly all interviewees. Small scale farming is used to grow vegetables for domestic use, but some respondents had plots of land where cash crop plantations (e.g. cashew) were grown. Other crops in the region are cultivated or exploited, such as rice, palm oil, oranges and papaya. Despite planting several species, every interviewee also used wild plants collected in the protected forest.

During the interviews, 120 items were recorded, with 82 species identified. The number of plants used by people from Cantanhez was higher than in Gola RNP (~ 8 wild plants). As seen in Gola, most people used wild plants in traditional medicine, food, and house and tool construction. Although access to health clinics is easier in Cantanhez due to a network of roads in the park, reliance on wild plants remained high. Differences in plant use were observed between age categories and community groups but not along with other demographic factors (further details in Chapter 2, section 3.2.3 and 3.3.2). People collected plants more often in the dry season and a portion of the harvested items were commercialised. Nevertheless, all interviewees used plants for domestic purposes. Cantanhez has different types of forest with varying vegetation composition, but respondents claimed to collect plants from most terrains in the park, including dense forest and mangroves. The protected or sacred forest, however, was a forbidden place or restricted access and/or harvesting conditions for some interviewees. Few respondents asked other members of the community to collect plants for them (5.4%), probably because the forest is relatively close to the settlements. The plants most frequently mentioned by respondents belong to the plant Families Leguminosae and Apocynaceae, which are some of the most abundant in the NP. Seven plants were identified as the most important for communities in Cantanhez possibly because the same plant can be used for several different purposes. Among those were *Dialium guineense*, *Parinari excelsa* and *Elaeis guineense* found in most habitats, *Anisophyllea laurina* found only in dense forest, *Cassia sieberiana* present in secondary forest and palm grove, and *Pterocarpus erinaceus* found in savannah.

5.3.3 Data collected in the field and produced in the laboratory

Primate faecal collection and subsequent plant DNA extraction and amplification allowed the identification of plants consumed by non-human primates. Interviews with people living in close proximity with non-human primates, permitted the inventorying of the most common wild plants harvested by humans. By comparing between the two databases the degree of plant overlap between all species was possible, and the most important plants for all and for each could be identified.

5.3.3.1 Biological data collection

Initially, faecal samples from the two primate species were collected in forest areas known to be used by the study species. Sampling took place at several sites to sample a high number of social groups dispersed across the protected area. Faecal matter was stored in the silica gel in the field, and primate and plant DNA was extracted later in the laboratory. Each sample was barcoded using specific primers to identify the primate species from which the faeces originated. Plant DNA was amplified using the universal plant marker ITS2 and sequenced under a high-throughput technique (DNA metabarcoding) in the Illumina MiSeq platform. Field and laboratory protocols are described in detail in Chapters 3 and 4.

5.3.3.2 Social data collection

Alongside non-invasive sample collection, structured interviews were conducted in villages nearby the sampling sites. The interviews were aimed at people from different demographic groups to broadly cover plant use by human communities. The interviews were conducted orally and included an initial section on the demography of the respondent, followed by closed and open-end questions on the use of plants collected in the protected forest. A freelisting section was included, where people would recall the wild plants they use for different purposes. Afterwards, information from the interviews was coded and treated to obtain qualitative and quantitative data for following analyses. The methodology used in this part of the work is found in Chapter 2.

5.3.4 Statistical analysis

5.3.4.1 Non-human primate diet

Dietary diversity of each primate was estimated using the Hill-diversity measure in R v4.1.3 (*R Core Team* 2022) with the package *iNEXT* (Hsieh et al. 2016). The efficiency of sample coverage (number of detections of plant DNA for each zOTU) was also assessed with the same software. Further details can be found in Chapter 3, section 3.3.4.2. To obtain the taxonomic detail on ~12 million plant sequencing reads generated in the Illumina V2 250bp paired-end MiSeq chip, the bioinformatics pipeline (modified from Drake et al. 2022) was performed using LINUX and comprised several programs and scripts written in BASH and PERL. The pipeline with all trimming, filtering and cleaning steps, and final taxonomic assignment is described in Chapter 3, section 3.3.4.1. High-throughput sequencing amplification success of western red colobus and chimpanzee samples was high (90.3%) and produced a final dataset with 171 plant taxa (61 in western red colobus diet, and 33 in chimpanzee diet). This result was obtained after applying a conservative approach to the taxonomic identification, which was chosen so plant diversity detected in the diet of primates would not be inflated. Due to the potential of the ITS2 marker to amplify fungal DNA, 3.4% of zOTUs were taxonomically assigned to fungi and 1.2% belonged to plant taxa absent in West Africa. Final tables indicating the plant taxa in each primate diet in each NP were produced and frequency of occurrence calculated (F_o% - number presences across all samples) for each plant item.

5.3.4.2 Spatial variation in primate diet

The two primates inhabiting the same protected forest in Sierra Leone and in Guinea-Bissau, consumed a portion of the plant species available. To compare the use of forest resources by sympatric primates in each NP, Levin's index of niche breadth (Levins 1968) was estimated for the primate taxa using the 'niche.width' function of the R package *spaa* (Zhang and Zhang 2013) and standardised with equation one from Razgour et al. (2011). Pianka's index of trophic niche overlap between primate taxa was quantified (0 = no overlap, 1 = complete overlap) and compared against "null model" randomisation tests for using the function 'niche.overlap.boot' in *spaa* (Gotelli 2000; Zhang and Zhang 2013). Mean plant richness (number of species per sample) was calculated for each primate species in each NP and compared using a nonparametric Kruskal-Wallis test to determine significant differences between means. To evaluate differences in the dietary composition of both primates in each NP, a model-based approach using simultaneous generalised linear models (function 'manyglm' in R package mvabund (Wang et al. 2012)), a binomial error family and respective link function was used, and implemented a Monte Carlo resampling.

5.3.4.3 Plant salience for humans in each site

For this chapter, the importance of wild plants across human communities in each NP was estimated using a Salience analysis (Smith 1993) conducted in R3.6.1 (*R Core Team* 2022) with the package AnthroTools (Jamieson-Lane and Purzycki 2016). In this analysis, I only used interviews from respondents that confirmed using wild plants collected in the protected area (N = 35 in Gola RNP; N = 56 in Cantanhez NP). To calculate the Salience of each item, the rank in which items were listed by each participant is used, as well as the total number of items cited in the dataset for each NP. To calculate the composite salience of each item across the dataset for each NP, the salience scores are added and divided by the total number of respondents interviewed, including those for which the item was not listed. Additional information on the analysis and description of the formula used can be found in Chapter 2, section 3.2.4.2.

For a final comparison between the plants in the diet of the two primates and those used by people, a table for each NP was constructed. Plant use overlap was visualised with network diagrams generated using the *bipartite* R package (v.2.15 Dormann et al. 2008; Dormann et al. 2014).

5.4 Results

5.4.1 Dietary diversity, niche indices and spatial variation

For the following analysis, only the datasets with samples collected in 2018 in Gola RNP and 2019 in Cantanhez NP were considered. A total of 416 western red colobus and western chimpanzee faecal samples were collected across both NPs. In Gola RNP, 207 samples were identified as *P. b. badius*, with 180 samples of red colobus collected in Gola Central and 27 in Gola South, and six were identified as *P. t. verus,* two chimpanzee samples in Gola Central and four in the Gola South. All samples were successfully sequenced. Sampling was sufficient to cover 98.1% (±0.9, CI 95%) of the *P. b. badius* dietary community (N = 207) but only 85.5% (±12.8, CI 95%) for *P. t verus*, probably due to the small sample size (N = 6). Despite the large area of suitable habitat for chimpanzees in Gola RNP, a small number of samples was collected probably due to the very low population densities in the park and difficulties to detect unhabituated individuals in the dense vegetation.

In Cantanhez NP, 101 samples were identified as *P. b temminckii* and 99 were collected in the south area of the park which is mainly composed of primary and secondary forests, and mangrove habitat (Fig. A.1.1 in Appendix 1). The other two samples were collected in areas where this species was thought to be absent (Áfia, north of the park). 56 samples were barcoded to *P. t. verus*, and 22 of those were sampled in the north of the park, where the vegetation is mostly grassland savannah. A total of 157 primate samples from Cantanhez NP were successfully amplified for the ITS2 primer. Hill-diversity estimates suggested that sampling in this park had a higher coverage of the dietary communities of both primates than in Gola RNP (*P. b. badius* = 98.6% ±0.9, CI 95%; *P. t. verus* = 95.9% ±2.6, CI 95%). The results implied that only 1.4% and 4.1% of plants in the theoretical diet of western red colobus and chimpanzees, respectively, were not detected in this study (Hill-diversity analysis explained in detail in Chapter 3, section 3.3.4.2).

Dietary richness varied significantly between primate species within the same NP and for the same primate between NPs (Table 5.1). Total plant richness in red colobus diet was higher in Gola RNP than in Cantanhez NP, despite botanical surveys providing similar number of recorded plants (899 species in Gola RNP, 863 species in Cantanhez NP). However, the mean number of plant taxa detected in the diet of *P. b. temminckii* was significantly higher than in *P. b. badius* (t = -3.37, df = 309.63, *p*-value < 0.001). The opposite result was observed in western chimpanzees' diet. Raw plant richness was higher in Cantanhez NP, but western chimpanzees in Gola RNP fed on fewer plants than in Cantanhez (W = 265.5, *p*-value = 0.02).

Table 5.1 Plant dietary richness across primate species and NPs.

	Gola RNP		**Cantanhez NP**	
	P. b. badius	*P. t. verus*	*P. b. temminckii*	*P. t. verus*
No. samples	207	6	101	56
Total plant taxa	62	10	45	24
Mean plant richness	4.19 (±1.66)	4.50 (±1.38)	5.33 (±1.88)	3.02 (±1.55)
Levin's niche breadth *	0.31	0.09	0.39	0.14

Levin's niche breadth adjusted using Razgour et al. (2011) equation

Variation in niche breadth and overlap was also observed for primate species in the NPs. Red colobus dietary breadth was generally higher than for chimpanzees. When comparing habitats, the niche breadth for *P. badius* and *P. t. verus* was wider in

Cantanhez than in Gola RNP (Table 5.1). In addition, niche overlap between the two primates was higher in Cantanhez NP than in Gola RNP (Table 5.2).

Table 5.2 Dietary niche overlap between sympatric primates, western chimpanzees and western red colobus, in Gola RNP and Cantanhez NP. Observed values were compared against "null model" randomisation tests to obtain the expected values of niche overlap (0 = no overlap, 1 = complete overlap).

Pianka's niche overlap	**Observed**	**Expected**	**Std**	**LCI**	**UCI**	**No. replicates**
Gola RNP	0.109	0.112	0.074	0.008	0.281	9999
Cantanhez NP	0.517	0.512	0.105	0.272	0.686	9999

Std: standard deviation; LCI: lower confidence interval at 95%; UCI: upper confidence interval at 95%; No. replicates: number of randomisations.

Assumptions of normally distributed data were not met for the model testing for differences in dietary composition between primate species in Gola RNP. This is likely a result of disparity in sample size (N*Pbb* = 207, N*Ptv* = 6), hence results from this test were discarded. A NMDS visualisation is included for illustrative purposes only, but a few outliers were removed simply for graphical reasons (NMDS stress value = 0.18) (Fig. 5.1). In Cantanhez NP, plant diet composition (at the zOTU level) differed significantly between primate species (N*Pbt* = 101, N*Ptv* = 56; LRT = 446.4, *p*-value < 0.001). A total of 19 plant taxa had significant GLMs (Table 5.3), explaining the variation observed. Dietary composition between primates was visualised using NMDS (Figure 5.2) but one chimpanzee sample was an outlier and removed when plotting the data (NMDS stress value = 0.16).

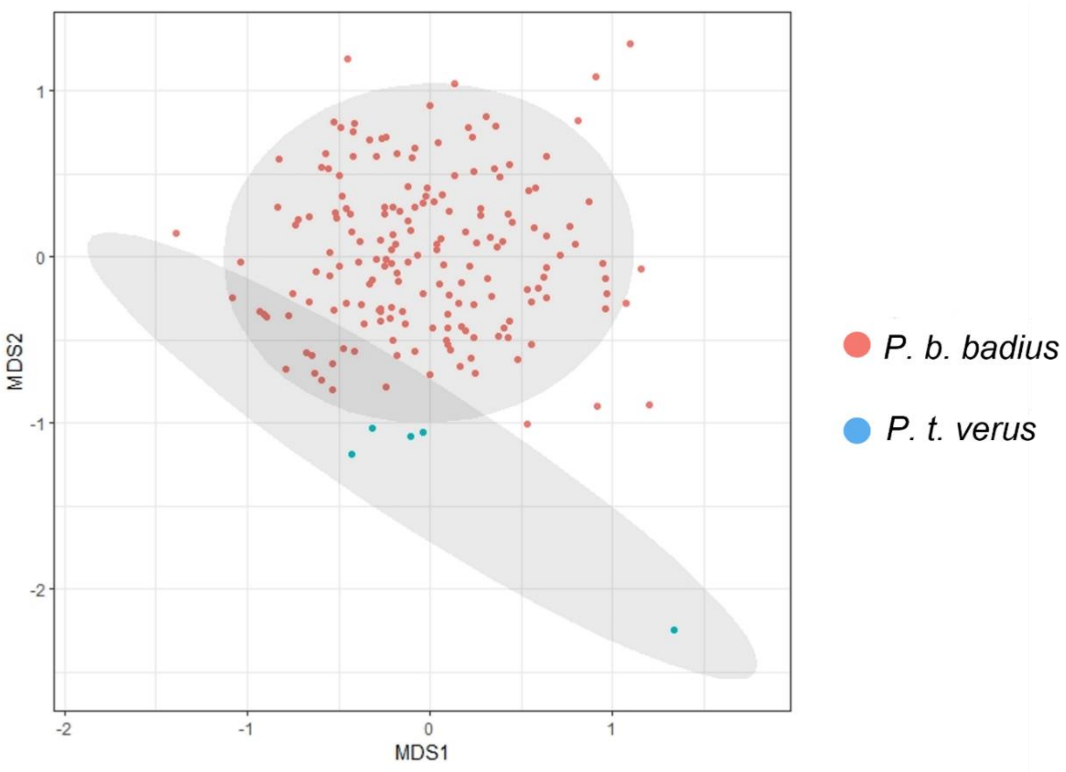


Figure 5.1 Diet composition variation in sympatric primates of Gola RNP. Differences in the diet composition of *P. t. verus* (blue dots, bottom elipse) and *P. b. badius* (red dots, top elipse) inhabiting the continuous and healthy forest of Gola are visualised using a NMDS analysis. Variation could not be assessed for statistical significance due to model not meeting the assumption of normally distributed data.

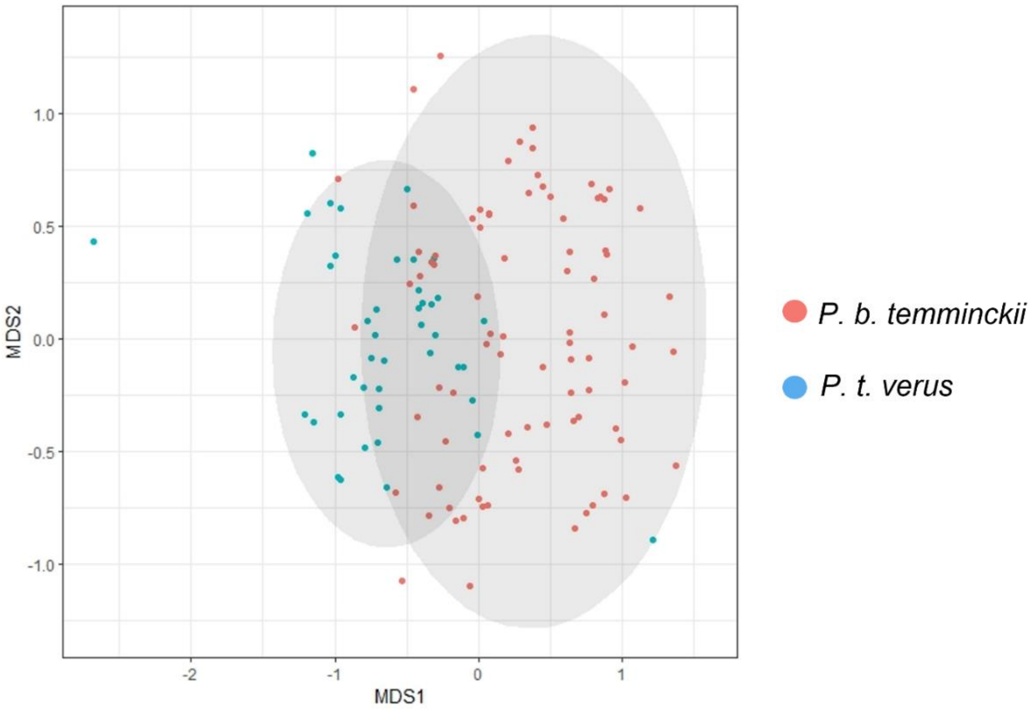


Figure 5.2 Diet composition variation in sympatric primates of Cantanhez NP. Diet compositions of *P. t. verus* (blue dots, left elipse) and *P. b. temminckii* (red dots, right elipse) inhabiting the fragmented and disturbed forest of Cantanhez are significantly different. This variation is visualised using a NMDS analysis.

5.4.2 Plants most consumed by non-human primates and crop feeding

Primates from Gola RNP fed on 64 plant taxa belonging to 34 plant Families. Across the combined dataset, the top five plant taxa that had a higher prevalence in the diet of primates in Gola RNP, were only detected in red colobus samples. These were from *Lophira alata* to *Nauclea* spp. on Table 5.3, which were detected in more than 20% of *P. b. badius*, with zero presence in *P. t. verus* samples. This is likely a result of the low number of chimpanzee faecal samples. Nevertheless, half of the plant taxa chimpanzees consumed were also consumed by red colobus (Fig. 5.3). *Ficus* spp. (F_o = 18.31%) was the taxa that contributed most to the diet of both non-human primates in Gola RNP and was eaten in both seasons, followed by *Manilkara obovata* (F_o = 13.62%) which was eaten in the rainy season by both non-human primates and in the dry season by red colobus only. All three other plants, *Myrianthus arboreus*, *Macaranga barteri* and *Musanga cecropioides*, had a frequency of occurrence lower than 6% in samples collected across the park.

Table 5.3 Frequency of occurrence of plant species consumed by non-human primates in Gola RNP. The 'Both primates' column is the frequency of occurrence, F_o (%), calculated across the combined dataset (western chimpanzees and western red colobus diet, N samples = 213; total number of plants consumed, including crops, N = 67). Values are shaded from darker (most frequently consumed item) to lighter colours (least consumed item, or not ingested). Plant family and taxa in bold are consumed by both primates.

		F_o(%)		
Plant Family	**Plant taxon**	**Both primates**	***P. b. badius***	***P. t. verus***
Ochnaceae	*Lophira alata*	36.15	37.20	0.00
Fabaceae	*Anthonotha macrophylla*	35.68	36.71	0.00
Combretaceae	*Combretum* spp.	32.86	33.82	0.00
Chrysobalanaceae	*Maranthes aubrevillei*	32.86	33.82	0.00
Rubiaceae	*Nauclea* spp.	20.66	21.26	0.00
Moraceae	***Ficus* spp.**	18.31	16.91	66.67
Clusiaceae	*Pentadesma butyracea*	16.90	17.39	0.00
Fabaceae	*Didelotia idea*	15.49	15.94	0.00
Euphorbiaceae	*Manniophyton fulvum*	15.02	15.46	0.00
Rubiaceae	*Keetia* spp.	14.55	14.98	0.00
Meliaceae	*Carapa procera*	14.08	14.49	0.00
Sapotaceae	***Manilkara obovata***	13.62	13.53	16.67
Dilleniaceae	*Tetracera* spp.	12.21	12.56	0.00
Meliaceae	*Trichilia monadelpha*	11.74	12.08	0.00
Myrtaceae	*Eugenia* spp.	11.27	11.59	0.00
Combretaceae	*Strephonema pseudocola*	10.80	11.11	0.00
Loganiaceae	*Strychnos* spp.	10.33	10.63	0.00
Combretaceae	*Terminalia* spp.	7.98	8.21	0.00
Phyllanthaceae	*Bridelia micrantha*	7.51	7.73	0.00
Fabaceae	*Cryptosepalum tetraphyllum*	7.04	7.25	0.00
Fabaceae	*Dalbergia* spp.	5.63	5.80	0.00
Urticaceae	***Myrianthus arboreus***	5.16	3.86	50.00
Fabaceae	*Daniellia ogea*	4.23	4.35	0.00
Meliaceae	***Macaranga barteri***	3.76	3.38	16.67
Euphorbiaceae	*Heckeldora leonensis*	3.76	3.86	0.00
Polygonaceae	***Musanga cecropioides***	3.29	0.48	100.00
Urticaceae	*Afrobrunnichia erecta*	3.29	3.38	0.00

Chrysobalanaceae	*Parinari* spp.	3.29	3.38	0.00
Melastomataceae	*Memecylon* spp.	2.82	2.90	0.00
Malvaceae	*Rhodognaphalon* spp.	2.82	2.90	0.00
Sterculiaceae	*Cola* spp.	2.35	2.42	0.00
Malpighiaceae	*Costus* spp.	1.88	0.00	66.67
Fabaceae	*Acridocarpus plagiopterus*	1.88	1.93	0.00
Fabaceae	*Aganope leucobotrya*	1.88	1.93	0.00
Rubiaceae	*Berlinia confusa*	1.88	1.93	0.00
Fabaceae	*Gilbertiodendron preussii*	1.88	1.93	0.00
Sapotaceae	*Omphalocarpum pachysteloides*	1.88	1.93	0.00
Fabaceae	*Paramacrolobium coeruleum*	1.88	1.93	0.00
Fabaceae	*Brachystegia leonensis*	1.41	1.45	0.00
Chrysobalanaceae	*Dactyladenia smeathmannii*	1.41	1.45	0.00
Rhizophoraceae	*Hypselodelphys violacea*	0.94	0.00	33.33
Fabaceae	*Anisophyllea meniaudi*	0.94	0.97	0.00
Marantaceae	*Helictonema velutinum*	0.94	0.97	0.00
Lecythidaceae	*Napoleonaea* spp.	0.94	0.97	0.00
Simaroubaceae	*Odyendea klaineana*	0.94	0.97	0.00
Lamiaceae	*Vitex* spp.	0.94	0.97	0.00
Celastraceae	*Gouania longipetala*	0.47	0.00	16.67
Sapindaceae	*Megaphrynium* spp.	0.47	0.00	16.67
Rubiaceae	*Campylostemon* spp.	0.47	0.48	0.00
Rubiaceae	*Chytranthus* spp.	0.47	0.48	0.00
Apocynaceae	*Craterispermum caudatum*	0.47	0.48	0.00
Clusiaceae	*Fleroya* spp.	0.47	0.48	0.00
Rhamnaceae	*Funtumia africana*	0.47	0.48	0.00
Rubiaceae	*Garcinia afzelli*	0.47	0.48	0.00
Fabaceae	*Hutchinsonia barbata*	0.47	0.48	0.00
Meliaceae	*Leptoderris* spp.	0.47	0.48	0.00
Euphorbiaceae	*Lovoa trichilioides*	0.47	0.48	0.00
Sapotaceae	*Macaranga heterophylla*	0.47	0.48	0.00
Marantaceae	*Malacantha alnifolia*	0.47	0.48	0.00
Rubiaceae	*Mussaenda* spp.	0.47	0.48	0.00
Fabaceae	*Plagiosiphon emarginatus*	0.47	0.48	0.00
Fabaceae	*Platysepalum hirsutum*	0.47	0.48	0.00
Menispermaceae	*Rhigiocarya racemifera*	0.47	0.48	0.00
Cucurbitaceae	*Ruthalicia eglandulosa*	0.47	0.48	0.00

The dietary analysis of primates in Cantanhez NP revealed a total of 48 plant taxa, belonging to 27 plant Families. Some wild plants are only consumed by one of the primate species, at different frequencies of occurrence (Table 5.3). Two plant taxa made the bulk of both primates' diet, being present in at least 41% of the samples. Leaves and fruit of *Ficus* spp. and *Treculia africana* were consumed by red colobus and chimpanzees, at high frequencies and in both seasons. These trees can be found in most forest types of Cantanhez. *P. b. temminckii* and *P. t. verus* diet overlapped in 15 wild plant taxa (Fig. 5.4). Some taxa were more frequently detected in red colobus diet (*Tetracera potatoria*, *Mezoneuron benthamianum* and *Trichilia prieuriana*) than in chimpanzees, but the opposite was also observed (e.g. *Neocarya macrophylla*, *Mussaenda elegans*).

Table 5.4 Frequency of occurrence of plant species consumed by non-human primates in Cantanhez NP. The 'Both primates' column is the frequency of occurrence, F_o (%), calculated across the combined dataset (western chimpanzees and western red colobus diet, N = 157; total number of plants consumed, including crops N = 52). Values are shaded from darker (most frequently consumed item) to lighter colours (least consumed item, or not ingested). Plant family and taxa in bold are consumed by both primates. Likelihood ration tests (LRT) and *p* values for the plant taxa having a significant impact on diet differentiation between primates, are presented.

		F_o(%)			**Univariate test**	
Plant Family	**Plant taxon**	**Both primates**	***P. b. temmin.***	***P. t. verus***	**LRT**	***p*-value**
Moraceae	***Ficus* spp.**	52.87	38.61	78.57	24.21	0.001
Moraceae	***Treculia africana***	41.40	51.49	23.21	12.37	0.005
Chrysobalanaceae	***Parinari excelsa***	29.30	39.60	10.71	16.16	0.002
Bombacaceae	***Ceiba pentandra***	26.11	15.84	44.64	15.05	0.002
Fabaceae	*Leptoderris* spp.	25.48	39.60	0.00	42.59	0.001
Sapotaceae	*Malacantha alnifolia*	22.29	34.65	0.00	36.26	0.001
Combretaceae	*Combretum* spp.	17.20	26.73	0.00	26.85	0.001
Rubiaceae	*Ixora* spp.	16.56	25.74	0.00	25.73	0.001
Moraceae	***Milicia regia***	16.56	12.87	23.21	-	-
Fabaceae	*Cassia sieberiana*	15.92	24.75	0.00	24.62	0.001
Euphorbiaceae	***Phyllanthus muellerianus***	15.29	12.87	19.64	-	-
Dilleniaceae	***Tetracera potatoria***	15.29	22.77	1.79	15.87	0.002
Avicenniaceae	*Avicennia germinans*	12.10	18.81	0.00	18.19	0.001
Hypericaceae	*Harungana madagascariensis*	11.46	17.82	0.00	17.15	0.002
Rubiaceae	*Uncaria africana*	10.83	16.83	0.00	16.12	0.002
Fabaceae	***Mezoneuron benthamianum***	10.19	14.85	1.79	8.49	0.034

Celastraceae	*Simicratea welwitschii*	8.92	13.86	0.00	13.10	0.004
Meliaceae	***Trichilia prieureana***	8.92	12.87	1.79	-	-
Fabaceae	*Dichrostachys cinerea*	8.28	12.87	0.00	12.11	0.005
Chrysobalanaceae	***Neocarya macrophylla***	7.01	1.98	16.07	10.67	0.012
Fabaceae	*Crudia senegalensis*	5.73	8.91	0.00	-	-
Rubiaceae	*Keetia venosa*	3.82	5.94	0.00	-	-
Sapotaceae	***Mimusops adongensis***	3.82	2.97	5.36	-	-
Meliaceae	*Trichilia monadelpha*	3.82	5.94	0.00	-	-
Fabaceae	***Dalbergia* spp.**	3.18	1.98	5.36	-	-
Rubiaceae	***Mussaenda elegans***	3.18	0.99	7.14	-	-
Olacaceae	*Ximenia americana*	3.18	4.95	0.00	-	-
Malpighiaceae	*Acridocarpus plagiopterus*	2.55	3.96	0.00	-	-
Rhizophoraceae	***Anisophyllea laurina***	2.55	0.99	5.36	-	-
Rubiaceae	*Coffea* spp.	2.55	3.96	0.00	-	-
Euphorbiaceae	*Macaranga heterophylla*	2.55	3.96	0.00	-	-
Simaroubaceae	***Odyendea klaineana***	2.55	2.97	1.79	-	-
Sapindaceae	*Paullinia pinnata*	2.55	3.96	0.00	-	-
Fabaceae	*Vigna* spp.	2.55	0.00	7.14	8.44	0.068
Meliaceae	*Carapa procera*	1.27	1.98	0.00	-	-
Rubiaceae	*Cremaspora triflora*	1.27	1.98	0.00	-	-
Fabaceae	*Pterocarpus* spp.	1.27	1.98	0.00	-	-
Celastraceae	*Reissantia indica*	1.27	1.98	0.00	-	-
Ulmaceae	*Trema orientalis*	1.27	0.00	3.57	-	-
Rubiaceae	*Tricalysia* spp.	1.27	1.98	0.00	-	-
Tiliaceae	*Triumfetta* spp.	1.27	0.00	3.57	-	-
Urticaceae	*Urera oblongifolia*	1.27	0.00	3.57	-	-
Vitaceae	*Ampelocissus leonensis*	0.64	0.00	1.79	-	-
Rutaceae	*Citrus limon*	0.64	0.00	1.79	-	-
Apocynaceae	*Cryptolepis sanguinolenta*	0.64	0.99	0.00	-	-
Malvaceae	*Hibiscus* spp.	0.64	0.99	0.00	-	-
Convolvulaceae	*Merremia* spp.	0.64	0.00	1.79	-	-
Rubiaceae	*Rothmannia whitfieldii*	0.64	0.99	0.00	-	-

5.4.3 Plant co-use in each protected area

Plant use by humans was evaluated in Chapter 2, and the results revealed a high use of wild plants in both NPs (Table 5.5 summarises results). People in the Gola region used a total of 44 plants while in Cantanhez diversity was higher, with respondents listing a total of 82 plant taxa. The mean number of plants listed per respondent were significantly different between the NPs (Table 5.4; W = 454.5, *p*-value <0.001). The plants with the highest salience for human communities living on the outskirts of Gola protected area were the trees bitter kola (*Garcinia kola*), chewing stick (*Massularia acuminata*), West African black pepper (*Piper guineense*) and laburnum (*Cassia sieberiana*), and two types of rattan palm (*Eremospatha* spp.). In Cantanhez, where villages are located inside the protected area, the most important wild plants were also trees such as Sierra-Leone tamarind (*Dialium guineense*), monkey apple (*Anisophyllea laurina*), rough-skinned plum (*Parinari excelsa*) and West African laburnum (*Cassia sieberiana*) among others (Table 2.6 in Chapter 2).

Table 5.5 Descriptive statistics of wild plants used by human communities in two NPs. The top plant species and respective families are presented. Local common names are in brackets.

	Gola RNP	**Cantanhez NP**
Total no. of items listed	49	120
Total no. plant species listed	44	82
Mean no. plants / respondent	4.86 ± 2.8	7.96 ± 3.4
Top plant Families listed	Fabaceae Rubiaceae Arecaceae	Fabaceae Apocynaceae -
Most salient plants (scientific and common names in Creole/English)	*Garcinia kola* (bitter kola) *Massularia acuminata* (bush ataya) *Eremospatha* spp. (rattan) *Piper guineense* (bush pepper) *Cassia sieberiana* (W. Africa laburnum)	*Dialium guineense* (veludo) *Anisophyllea laurina* (miséria) *Parinari excelsa* (manpatace) *Cassia sieberiana* (canafistra) *Elaeis guineensis* (palmera)

In this study, non-human primates consumed a high diversity of wild plants (sections 5.4.1 and 5.4.2) but also integrated food items cultivated by local communities in their diet. In Gola RNP, three crops were consumed by primates, but none were common across the two primates. All were detected at low frequencies, with chimpanzees feeding on *Abelmoschus* spp., and red colobus on *Coffea* spp. and

Enterolobium spp. (Table 5.6). Of the four cultivated food items detected in the diet of primates from Cantanhez NP, all were consumed by red colobus but only two by chimpanzees. However, frequencies of occurrence were considerably higher in chimpanzee samples (Table 5.6). Cashew (*Annacardium occidentale*) had a higher frequency in *P. t. verus* samples than in *P. b. temminckii* (8.93% and 0.99%, respectively), but mango (*Mangifera indica*) was the preferred crop by both, yet with higher frequency in chimpanzees ($F_o = 23.21\%$, compared to 8.91% in red colobus). It is possible that the presence of *Coffea* spp. in *P. b. temminckii* diet might be a contamination from red colobus samples from Gola RNP, as the plant, though native to Guinea-Bissau, has not been recorded in any of the park botanical surveys nor reported as cultivated in the region. However, Cantanhez red colobus have the same number of dietary counts as red colobus in Gola RNP (N = 4), which could imply that the result in Gola could also be a contamination. Nevertheless, in both study sites samples containing *Coffea* spp. were collected in different season, and in Gola, in different transects. This outcome requires further analysis to understand if *P. badius* are truly consuming coffee plant parts (e.g. amplify DNA samples individually with a primer specific to *Coffea* spp.).

Table 5.6 Crops detected in *P. t. verus* and *P. badius* diet in each NPs. The 'Both primates' column is the frequency of occurrence, F_o (%) of each plant detected in western chimpanzees and western red colobus diet faeces collected in each NP.

				F_o(%)		
National Park	Plant Family	Plant taxon	Common name	Both primates	*P. badius*	*P. t. verus*
Gola	Malvaceae	*Abelmoschus* spp.	-	1.88	0.00	66.67
	Rubiaceae	*Coffea spp.*	-	1.88	1.93	0.00
	Fabaceae	*Enterolobium* spp.	-	0.94	0.97	0.00
Cantanhez	Anacardiaceae	*Mangifera indica*	Mango	14.01	8.91	23.21
	Anacardiaceae	*Anacardium occidentale*	Cashew	3.82	0.99	8.93
	Solanaceae	*Solanum* spp.	-	1.27	1.98	0.00
	Cucurbitaceae	*Cucumis* spp.	-	0.64	0.99	0.00

Human communities living around and inside the NPs shared plant resources with the other primates of focus in this study. Co-use of wild plants in both NPs was more frequently observed between humans and red colobus than between humans and chimpanzees (Figures 5.3 and 5.4). Resource overlap was also greater in Cantanhez NP (8.9%) than in Gola RNP (3.7%) and this trend was still observed when crops were included (12.2% and 6.5%, respectively) (Table 5.9). In the continuous forest of Gola, one plant (*Macaranga barteri*) was commonly used by all three species, but with low frequency of occurrence in the diet of red colobus and low salience for humans (Table 5.7). Another plant taxa that was present in four of the six chimpanzee samples in Gola, and that it is likely cultivated by some of the local communities, was *Abelmoschus* spp.. However, it was not possible to identify which of the ~12 accepted species the zOTU corresponded to. *Carapa procera* was a species frequently detected in *P. b. badius* diet (F_o = 14.5%) and it was the 8th most salient plant for people in Gola RNP (Table with all plants consumed by primates and used by humans, can be found in Table A.5.1 in Appendix 5).

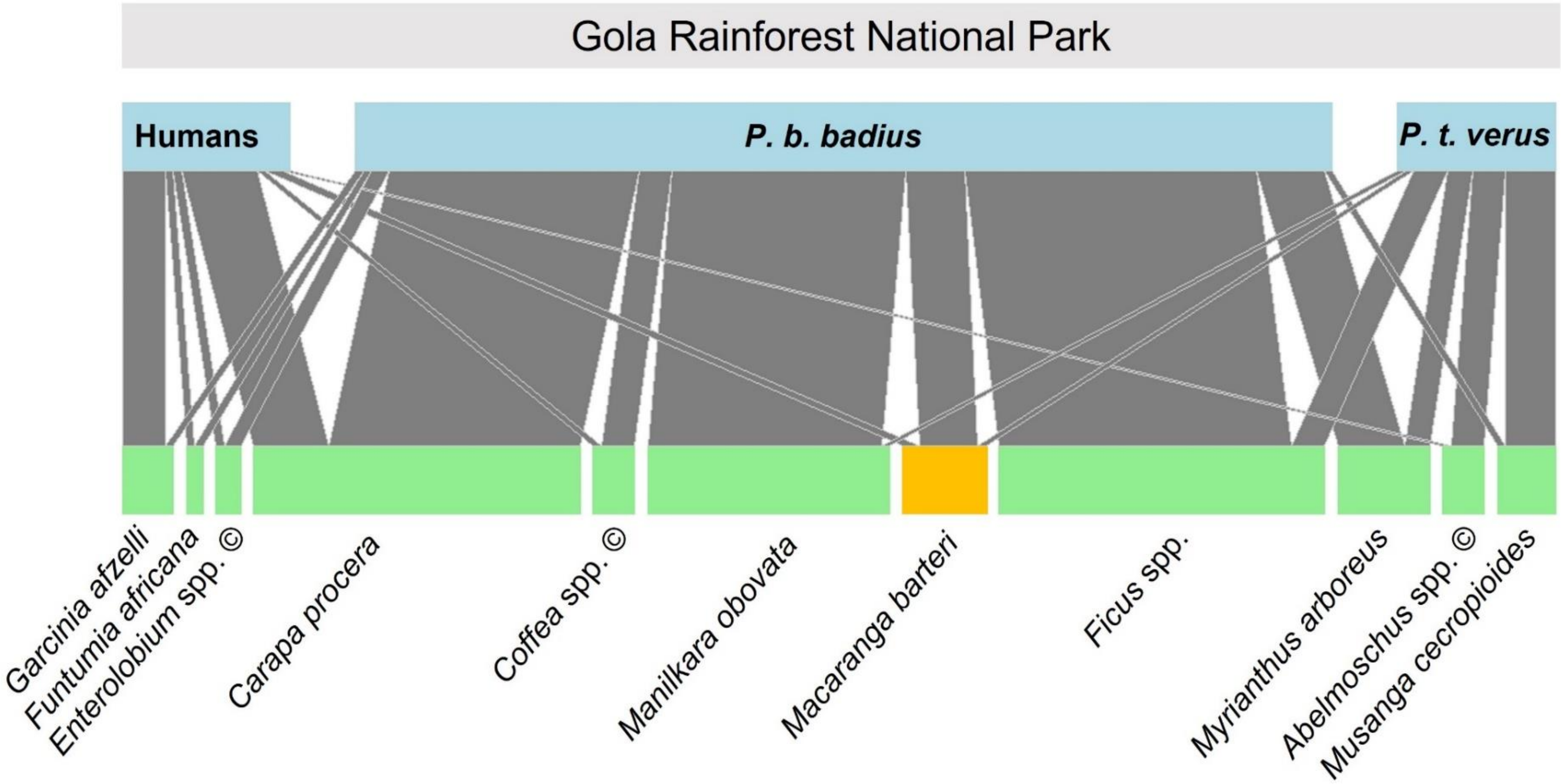


Figure 5.3 Network diagram showing the diet overlap between sympatric humans and non-human primates in Gola RNP. *Macaranga barteri* (highlighted in orange) was the only plant species co-used by all three groups. Thickness of interaction bar links represent the number of faeces for which the plant taxa was detected, and for humans, the number of times this plant was mentioned in the freelisting section of the interview. © Crops. Links from humans to crops are not representative of the number of times the plant was mentioned as this information was not collected in the interviews. However, crops are cultivated by humans, so there is an overlap between human use and plant in the diet of primates.

Table 5.7 List of wild plants and crops co-utilised in Gola RNP. Frequency of occurrence or salience score for items co-utilised by two or all three groups in Gola RNP.

	F_o(%)		Salience
Plant taxon	***P. b. badius***	***P. t. verus***	**Humans**
Abelmoschus spp. ©	0.000	66.667	Na
Carapa procera ~	14.493	0.000	0.134
Coffea spp. ©	1.932	0.000	Na
Enterolobium spp. ©	0.966	0.000	Na
Ficus spp. ^	16.908	66.667	0.000
Funtumia 224bovate224 ~	0.483	0.000	0.014
Garcinia afzelii ~	0.483	0.000	0.074
Macaranga barteri *	3.382	16.667	0.022
Manilkara 224bovate ^	13.527	16.667	0.000
Musanga cecropioides ^	0.483	100.000	0.000
Myrianthus arboreus ^	3.865	50.000	0.000

Wild plant taxa overlap between: * two primates and humans (N = 1), ~ only red colobus and humans (N = 3), ° only chimpanzees and humans (N = 0), and ^ only both primates (N = 4). © - introduced or native plant cultivated by humans and consumed by primates (N = 3: *P. b. badius* N = 2 for, *P. t. verus* N = 1).

In Cantanhez NP, six plants were consumed/used by both non-human primates and humans, with various frequencies of occurrence and Salience values (Table 5.8). In general, plants that were frequently detected in primates' diet had a low importance for humans, and vice-versa. The one exception was *Parinari excelsa*, which was detected in 39.4% of red colobus faecal samples and 10.7% of chimpanzee samples, and occupied the 3rd place in the salience list for plant use among the human communities in the park. These commonly used plants are mostly trees, found in all types of forests and consumed by primates in both seasons, except *Anisophyllea laurina* which was only detected in faecal samples collected in the dry season. Red colobus and humans alone shared four plant taxa, with *Cassia sieberiana* being the most important for both (F_o *P. b. temminckii* = 24.75%; 4th most salient item for humans). Humans and chimpanzees shared only one plant between the two (*Trema orientalis*), and it had low frequency of occurrence in chimpanzee diet (3.57%) but was in the top third most salient plants for humans (Salience = 0.046 ≈ 26th place in Salience list).

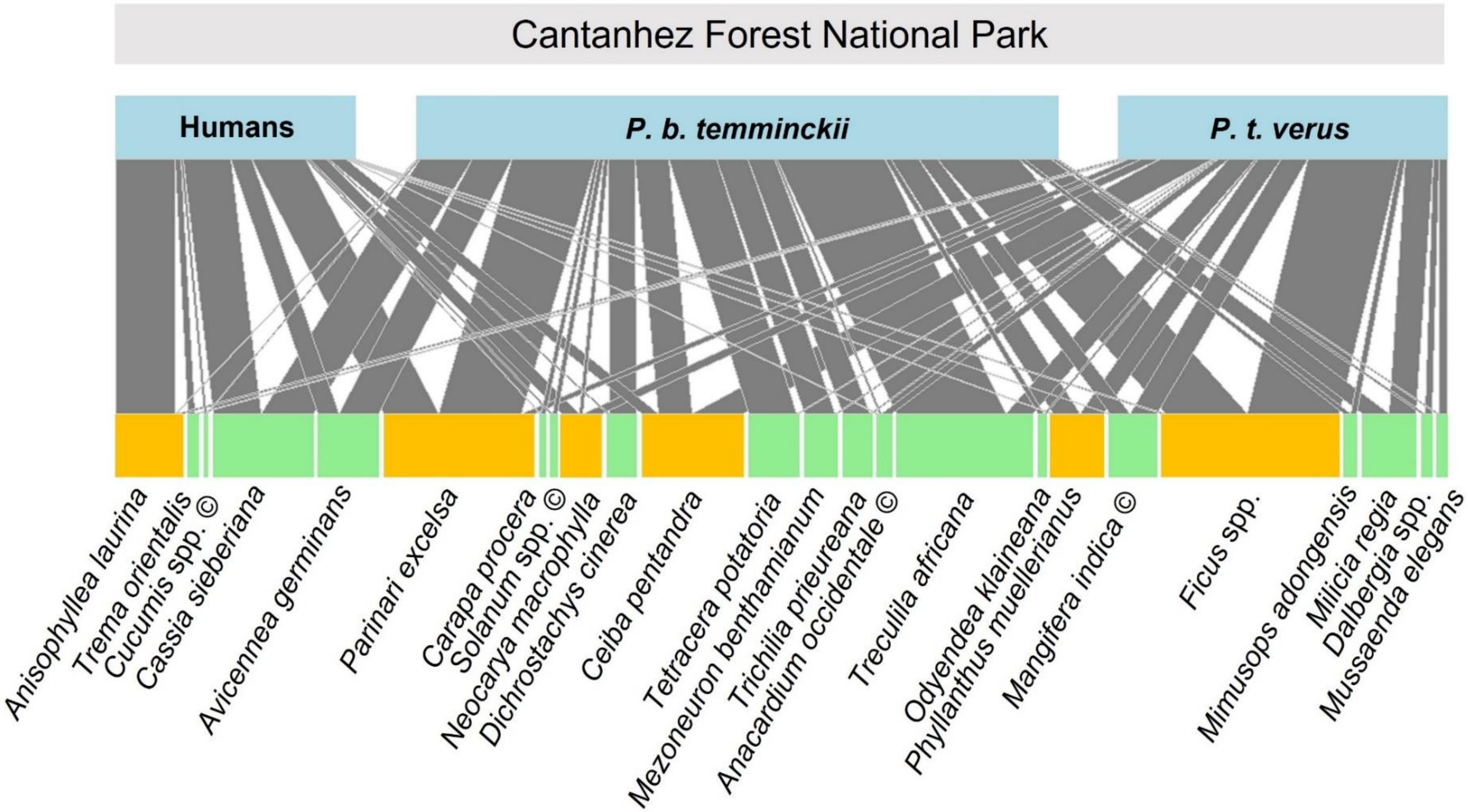


Figure 5.4 Network diagram showing the diet overlap between sympatric humans and non-human primates in Cantanhez NP. Six plant taxa (highlighted in orange) were co-used by all three groups, with higher use of *Ficus* spp., *Parinari excelsa* and *Ceiba pentandra*. Thickness of interaction bar links represent the number of faeces for which the plant taxa was detected, and for humans, the number of times this plant was mentioned in the freelisting section of the interview. © Crops. Links from humans to crops are not representative of the number of times the plant was mentioned as this information was not collected in the interviews. However, crops are cultivated by humans, so there is an overlap between human use and plant in the diet of primates.

Table 5.8 List of wild plants and crops co-utilised in Cantanhez NP. Frequency of occurrence or salience score for items co-utilised by two or all three groups in Gola RNP.

	F_o(%)		**Salience**
Plant taxon	***P. b. temminckii***	***P. t. verus***	**Humans**
Anacardium occidentale ©	0.990	8.929	na
Anisophyllea laurina *	0.990	5.357	0.348
Avicennia germinans ~	18.812	0.000	0.093
Carapa procera ~	1.980	0.000	0.007
Cassia sieberiana ~	24.752	0.000	0.255
Ceiba pentandra *	15.842	44.643	0.080
Cucumis spp. ©	0.990	0.000	na
Dalbergia spp. ^	1.980	5.357	0.000
Dichrostachys cinerea ~	12.871	0.000	0.006
Ficus spp. *	38.614	78.571	0.009
Mangifera indica ©	8.911	23.214	na
Mezoneuron benthamianum ^	14.851	1.786	0.000
Milicia regia ^	12.871	23.214	0.000
Mimusops adongensis ^	2.970	5.357	0.000
Mussaenda elegans ^	0.990	7.143	0.000
Neocarya macrophylla *	1.980	16.071	0.058
Odyendea klaineana ^	2.970	1.786	0.000
Parinari excelsa *	39.604	10.714	0.324
Phyllanthus muellerianus *	12.871	19.643	0.006
Solanum spp. ©	1.980	0.000	na
Tetracera potatoria ^	22.772	1.786	0.000
Treculia africana ^	51.485	23.214	0.000
Trema orientalis °	0.000	3.571	0.046
Trichilia prieureana ^	12.871	1.786	0.000

Wild plant taxa overlap between: * two primates and humans (N = 6), ~ only red colobus and humans (N = 4), ° only chimpanzees and humans (N = 1), and ^ only both primates (N = 9). © - introduced or native plant cultivated by humans and consumed by primates (N = 4: both primates N = 2, *P. b. temminckii* N = 2, *P. t. verus* N = 0).

Even though in the interviews we only asked information on wild plants used by people, the crops found in the non-human primate's diet are cultivated and used by humans. Hence, data on wild plants and crops can be incorporated and analysed. The co-use of all plants among the two primates and humans in each NP is summarised in Tables A.5.1 and A.5.2. Across the whole dataset, western chimpazees consumed fewer wild plants and crops in common with humans than the western red colobus.

Additionally, the total number of wild plants being consumed/used by both or either non-human primate, and humans was always higher in the altered habitat of Cantanhez NP than in the dense and continuous forest of Gola RNP. Overall, the level of wild and cultivated plant co-use in Cantanhez (12.2%) was nearly twice as high as that observed for Gola RNP (6.5%), but this difference is more accentuated when only wild plants are considered (co-use in Gola = 3.7%; co-use in Cantanhez = 8.9%).

Table 5.9 Levels of overlap in each NP, and per co-existing pair. Total number of plants and percentage of overlap was calculated for wild plants and crops only, and all plants, across each pair of co-users for each NP.

	Gola RNP (Tt taxa = 107)		**Cantanhez NP (Tt taxa = 123)**	
	Total no. plant taxa	**Overlap (%)**	**Total no. plant taxa**	**Overlap (%)**
Wild plant co-use (%) between:				
both primates and humans	1	0.93	6	4.88
western red colobus and humans	4	3.74	10	8.13
western chimpanzees and humans	1	0.93	7	5.69
Total wild plant overlap	**4**	**3.74**	**11**	**8.94**
Crop consumption by:				
both primates	0	0.00	2	1.63
western red colobus	2	1.87	4	3.25
western chimpanzees	1	0.93	2	1.63
Total crops overlap	**3**	**2.80**	**4**	**3.25**
Total co-use of wild plants and crops by:				
both primates and humans	1	0.93	8	6.50
western red colobus and humans	6	5.61	14	11.38
western chimpanzees and humans	2	1.87	9	7.32
Total overlap (wild plants and crops)	**7**	**6.54**	**15**	**12.20**

Tt taxa: total number of taxa identified from cited items in interviews to people, and food items detected in dietary analysis of primate faecal samples.

5.5 Discussion

The results of this cross-disciplinary research can offer a broader understanding of the co-utilisation of plants by humans and two primates living in or near protected areas, with different levels of anthropogenic pressure. Overall, primates fed on a wide range of wild plants, incorporating a few cultivated items in their diet, and local human communities cited more than 40 plants used for different purposes. As predicted, a higher degree of wild plant use overlap was observed for humans and primates living in the fragmented forest of Cantanhez NP, where the degree of human activities and land transformed into agricultural production is greater. Furthermore, crop consumption by primates was also more prevalent in Cantanhez than in Gola RNP.

5.5.1 Dietary patterns of sympatric primates within each national park

The primate feeding ecology literature shows great intra- and inter-species variation, demonstrating clearly that caution is required when interpreting results and drawing conclusions in this comparative study. Strushaker (2010) stated that intertaxa variation is difficult to distinguish from intrataxon and intragroup temporal variation, due to the pronounced interannual variation in diet and the different methods applied, especially in studies that last one year or less. Notwithstanding this caveat, comparisons between primate taxa within each NP and between NPs were conducted to assess the dietary flexibility of western red colobus (*P. badius*) and western chimpanzee (*P. t. verus*) to a more anthropogenically impacted landscape, Cantanhez NP in Guinea-Bissau.

Dietary composition varied significantly between *P. badius* and *P. t. verus* in Cantanhez NP, as expected given their specialised diet. Even though the length of sampling for this study was restricted to a few months in each season, longer sampling is likely to have resulted in similar patterns of variation. For example in an investigation conducted in Kibale NP, Uganda, comparing the diets of chimpanzees, blue monkeys, redtail monkeys and grey-cheeked mangabeys, researchers identified divergences in food items consumed by apes and monkeys when ripe fruit was scarce

(Wrangham et al. 1998). For a discussion on the seasonality effect on each primate species food choice, see Chapters 3 and 4.

Chimpanzees consumed larger quantities of ripe fruit which is more available during the dry season (December to April), while red colobus explored a wider range of food items (e.g. seeds, unripe fruit, leaves) which are available most of the year. Young foliage has two peaks of production in both NPs, one in the early dry season and one in the early rainy season, but mature leaves are constantly available and are a food item consumed by red colobus when other preferred items are scarce. In Gola RNP, though statistical tests could not be performed due to the large difference in sample size between primate species, one plant had marked differences in frequency of occurrence. The evergreen umbrella tree (*Musanga cecropioides*) was consumed by only one red colobus individual in the dry season, while all six chimpanzees fed on this species, some in the rainy and some in the dry season. This is likely because the umbrella fruit is available throughout most of the year, and is eaten by chimpanzees also in times of fruit scarcity (Yamakoshi 1998; Takemoto 2003), which would be in the rainy season in Gola RNP. Furthermore, the umbrella tree is a fast-growing and short-lived species that occupies secondary forests and old farms in closed forest (CJB 2022), a habitat that chimpanzees are more flexible to utilise than red colobus. This is the type of forest where primate samples containing *M. cecropioides* originated from: a secondary forest in Gola Central where old intense logging took place, and the community forest in Gola South where old farms are surrounded by forest resembling primary forest. In Cantanhez NP, the significant variation in dietary composition could be better explained by a much larger number of plants, but the pattern observed was similar. In some cases, plants that had a high frequency of occurrence in one primate species diet, had a low frequency or were not consumed at all by the other primate species. These plants were present in most habitats in the park, with two main exceptions. One was *Ceiba pentandra*, which is commonly found in secondary forest and was highly detected in chimpanzees' diet. The other plant (*Malacantha alnifolia*), present in nearly 35% of red colobus samples and absent from chimpanzee faeces, is also abundant in secondary forests and in palm groves (Catarino and Palminha 2018). This shows that red colobus in Cantanhez NP have some dietary flexibility allowing them to move through and feed in areas described as less suitable for the species.

Patterns of dietary richness observed among the two non-human primates in each NP support the literature and prediction 1. In this study, dietary diversity for both populations of the fruit specialist western chimpanzee were lower than for red colobus populations. This species richness difference between apes and monkeys has been observed previously, especially in a study comparing sympatric western gorilla and mangabey diet (Doran-Sheehy et al. 2006). Both species were frugivorous but in the forests of Central African Republic and the Republic of Congo, gorillas fed mainly on ripe fruit whereas mangabeys broadened their diet by eating several species that produced fruit or seeds in unripe, ripe and rotting fruits. Dietary breadth further supported the evidence of differences in diet between chimpanzees and red colobus, as estimates were higher for colobines, indicating a wider variance in resource use than chimpanzees. These results are probably related to the strategies adapted by each primate species when most common food items in their diet is reduced due to natural or anthropogenic causes. Because chimpanzees are ripe fruit specialists, they would rather travel further and for longer periods to find ripe over unripe fruit (Doran 1997; Rogers et al. 2004). On the other hand, if red colobus can meet their daily requirements with food items available in the area, which are more dependent on food quantity rather than quality compared to omnivorous or frugivorous primates, than they would only travel the necessary distance (Struhsaker 2010).

5.5.2 Intra and interspecies dietary variation between different landscapes

Overall, the results demonstrate that dietary diversity of available primate food in the degraded forest of Cantanhez NP is lower than the one in Gola RNP. The total number of plants detected in both primates diet was higher in Gola, suggesting a richer flora. While *P. t. verus* fed on average on fewer plant taxa in Cantanhez, *P. badius* increased the number of plants in their diet. As mentioned, quality is more important than quantity for frugivorous species, and this may be the reason for the significant difference between the two species. Red colobus and chimpanzees also broadened their dietary niche in the more heterogeneous landscape of Cantanhez NP, an unexpected result from what was proposed in prediction 2 based on the assumption that co-existing species would narrow their dietary niche in response to reduced food availability (Colwell and Futuyma 1971). Most likely, individuals from both primate species had a wider niche to meet their daily nutrients requirements, again supporting the idea that these animals are dietarily flexible. Comparing with chimpanzees, red colobus presented a wider dietary niche. In a cross-species analysis of habitat and dietary niche breadth, Swihart et al. (2003) determined that vertebrates with greater niche scores were the most tolerant species to habitat alteration. The result suggests that red colobus may respond better to changes in food availability possibly due to the ability to feed on a broader array of food items. Again, the dietary nature of chimpanzees could explain the difference observed, as the abundance of fruit may decrease in disturbed forests, whereas the availability of new leaves may not (Johns and Skorupa 1987). However, wide niche measure could simply be a result of greater variation within individuals' diet (Tinker et al. 2008). Red colobus have very small home ranges and daily travel distances (DTD) when compared with chimpanzees (red colobus home range = 0.038-0.57 km^2, DTD = 0.3-1.1 km (Struhsaker 2010); chimpanzees home range = 8-89 km^2, DTD = 0.5-9 km (Herbinger et al. 2001; Pruetz 2018; Vieira et al. 2019)), so the food consumed by each group may differ greatly, increasing the dietary niche. In Cantanhez, the heterogeneity of the habitat may contribute to greater within species variation, while in Gola RNP this variation might be attenuated. Due to small sample sizes per forest patch in Cantanhez, and for chimpanzees in Gola RNP it was not possible to conduct a statistical analysis of differences. However, comparisons of diet composition between groups of primates sampled in each forest block can be visualised in Figures A.5.1 and A.5.2 in Appendix 5. Significant differences in the diet of *P. b. badius* of Gola central and individuals in Gola south were observed, but this is likely a consequence of seasonal variation as samples in Gola south were only collected in the rainy season.

As sympatric non-human primates, western red colobus and western chimpanzees partially feed on the same plant species. A good example of a resource that is commonly consumed by apes, cercopithecines, colobines and other primates, and that was frequently detected in the diet of the two study species in both NPs, is the genus *Ficus* (Doran 1997; Davies et al. 1999; Struhsaker 2010; Hohmann et al. 2012; Bessa et al. 2015b). This does not mean, however, that they consume the exact same plant parts. In fact, red colobus prefer mostly young and mature leaves, and unripe figs, while chimpanzees prioritise ripe fruits. Some exceptions detected in our dataset might be *Parinari excelsa*, *Dalbergia* spp., *Tetracera potatoria* and *Mezoneuron benthamianum*, as both primates feed on leaves. In Gola RNP, only a small portion of the plants were commonly consumed by red colobus and chimpanzees, with low estimates for the magnitude of dietary overlap. Yet, the far greater resource overlap between primates observed in Cantanhez NP, suggests that some of the common plant taxa are key in the diet of both. Indeed, nearly one third of the total plant taxa identified in their diets were eaten by both primates, with six plants having relatively high frequencies of occurrence in both species' faeces (e.g. *Treculia africana*, *Ceiba pentandra*). This could mean, as in prediction 2, that plant diversity and availability in Cantanhez NP forest is lower, driving the two sympatric primates to share more food resources, or simply reflects a difference in the use of habitat type, as *T. africana* is found in dense forest and fallows.

The number of cultivars detected in the diet of primates in Cantanhez was twice as high as in Gola RNP. This result partly supports prediction 3: primates living in habitats with higher exposure to agriculture, as is the case of the agroforest mosaic of Cantanhez, would consume more crops as a response to habitat changes. As in a review exploring the patterns of cultivar consumption by chimpanzees, populations living in heavily disturbed forest-farm mosaic with higher exposure to crops, showed the greatest range of crops eaten (Hockings and McLennan 2012). Although it was not possible to specify the species of some crops consumed by primates, it is mostly certain that some species are farmed intensely and are more economically important to human populations than other crops. This was the case of coffee (*Coffea* spp.) consumed by red colobus in Gola RNP, and cashew (*Anaccardium occidentale*) eaten by both primates in Cantanhez NP. The other plant taxa identified as crops may have been obtained from abandoned or naturalised sources which were or still are exploited by people, such as *Mangifera indica* which is most likely encountered in fallows in Cantanhez NP and was strongly present in the diet of both primates. However, unexpectedly, the number of crops consumed by red colobus was higher than that for chimpanzees in both NPs (not supporting the second half of prediction 3). This result could be explained by the type of information it is reported in the literature. In many dietary studies, cultivated plants are not part of the natural diet and are not included in the data. In addition, because chimpanzees are perceived as species that can cause damage to economically important plants cultivated by people (Humle and Hill 2016), more research has been conducted for this species and in this field. Furthermore, red colobus in Tanzania visited the plantations in early morning or in the evening, when humans were not present (Kibaja 2014), and as many dietary studies are based on direct observations, many crop feeding events may be missed during field work on red colobus feeding ecology.

5.5.3 Plant overlap between three forest resource users

As anticipated (first part of prediction 4), overlap of plant resources between sympatric primates and humans was much more evident in Cantanhez NP than in Gola RNP, most likely due to the nature of the forest. In Gola RNP, the number of wild plants used by people was lower than in Cantanhez NP, but the number of plant taxa detected in primate diet was higher. This is likely a result of the low human intervention in the forest with very low levels of land transformation, allowing for high plant diversity. A detailed discussion of people's use of forest resources in both NPs can be found in Chapter 2. Resource co-use, measured as the proportion of common plants detected in primate faeces, using a DNA metabarcoding approach, and cited by people in structured interviews, showed that nearly 12.2% of all plants recorded across Cantanhez NP dataset, are co-utilised by the two primates and humans. This estimate was nearly two times larger than the overlap observed in Gola RNP, but the small number of samples collected for chimpanzees could be the reason for such a discrepancy between the two NPs. Nonetheless, when analysing the overlap between humans and each primate species separately, where sample size should not be an issue for red colobus, the same trend in resource co-sharing was observed. Regardless of sample size, a higher plant overlap would be expected in sympatric humans and non-human primates in Cantanhez NP than in Gola RNP because people are more exposed to the wild plants and primates to cultivated species, leading to more resource share between the two groups.

Humans in Cantanhez NP shared around 8% of the same plants with *P. b. temminckii* and ~6% with *P. t. verus*. Percentages of plant overlap for humans and the two primates in Gola RNP were lower, but red colobus still shared more plants with humans than chimpanzees. This result did not support the second part of prediction 4, where chimpanzees were expected to have a higher plant co-use with humans than red colobus. This trend did not change when crops were included in the analysis, as red colobus also fed on more cultivated species than chimpanzees. Since not many studies have been conducted on the co-use of plants by sympatric primates and humans, it is difficult to understand if these levels of co-use and differences would be expected or not. Nevertheless, tonkean macaques and humans co-utilised 17 tree species out of the 113 utilised by humans (Riley 2007), and the same number of naturally occurring plants was eaten by humans and chimpanzees at Fongoli, Senegal (Pruetz 2002 in Hockings and Humle 2009). A nine month study conducted in two forest fragments of Cantanhez NP (Caiquene and Cadique), identified fruit overlap between humans and chimpanzees from 27 wild plant species (Hockings et al. 2020). In comparison, our result for the same NP shows a lower number of co-used plants, with only seven wild plants (nine if crops are included) being used by both humans and chimpanzees. Due to the lack of information on the combined number of plants recorded in primate diet and used by people in the two previous studies, an overlap percentage cannot be estimated and compared with the results of the current study. Hence, the apparently lower overlap detected for this research could be explained by the shorter sampling months, and the lower depth of the information obtained in the interviews to local people. Differences in the technique used to characterise the diet of chimpanzees in both studies is explored in Chapter 3, but the lower species diversity detected in this study could also be due to technical factors and decisions taken upon the data production with the DNA metabarcoding approach. More limitations to this research are further evaluated in the next section.

The only plant utilised by all sympatric humans and primates in Gola RNP was *Macaranga barteri*, but although used in multiple treatments in Sierra Leone (sexually transmitted infections, as a laxative and haematinic (Asante-Kwatia et al. 2019)), the species had low salience for humans. The plant was also detected at low frequencies in *P. t. verus* and *P. b. badius* diet, similarly to Temminck's red colobus in Senegal where leaves contributed to 17% of the total diet (Wilkins 2017). This plant, together with *Carapa procera* which was highly salient for people and frequently detected in red colobus faeces, were common in the old logged areas of Gola South and wester Gola Central, probably explaining the co-use between all three groups. In Cantanhez NP, of the six co-utilised plants, *Parinari excelsa* was the one with highest overlap results. This tree was one of the most salient species for humans, highly important for red colobus in Cantanhez NP, and although detected at a lower frequency in the diet of chimpanzees, 10% of the samples still contained DNA from this plant. In Guinea-Bissau, humans used *P. excelsa* for almost all purposes: food, fuel, construction, and medicine (Catarino et al. 2020). The wood of the tree is also used to make barrels, drums and mortars (*PROTA4U* 2022). Generally, young leaves and leaf buds of *P. excelsa* are eaten by *Piliocolobus*, especially in areas where this tree is abundant, as is the case of Cantanhez NP (Scott 1992). Furthermore, as reported previously for the Cantanhez population, fruit traces of this tree were present in ~14% of the faeces collected in Hockings et al. (2020) study, and it was also one of the plants commonly used by *P. t. verus* and humans. Contrary to what was observed in Gola RNP, this wild plant is commonly found in primary forest and disturbed habitats (secondary forest and traditional orchards). Indication that humans and primates used the same wild plant in the same habitat during this study, would have required direct observations and more detailed interviews, reinforcing the importance of integrative approaches when studying complex species in complex social-ecological systems.

5.5.4 Study limitations

The degree of overlap detected in this study is likely underestimated. Single studies on the use of plants by humans in both NPs (Frazão-Moreira 2009; Munro et al. 2013; Jusu and Sanchez 2014), as well as dietary studies on the same or other species of red colobus and chimpanzees indicate higher species diversity (Yamakoshi 1998; Davies et al. 1999; Bessa et al. 2015b; Struhsaker 2017), which could result in greater wild and cultivated plant co-use between sympatric humans and primates. The need for longer sampling periods and at more time points in the year to overcome seasonal bias in people's answers and primate consumption is crucial. This strategy would increase sample size, which in turn would cover more primate groups to overcome intraspecific variation and would allow for more detailed information and better taxonomic identification of plants used by people. Even though sample size was considered in species richness and niche breadth calculations in this study, results need to be interpreted carefully because sample size for Gola chimpanzee population is relatively low. The aid of a botanist or an expert in local flora when conducting interviews would have most likely reduced the number of plants which could not be identified to the species.

Unexpectedly, chimpanzees showed lower levels of plant co-use with humans than red colobus. This could be an authentic outcome, or it could be due to technicalities during field and laboratory work, which did not allow for an exhaustive list of dietary taxa. The choice of method to obtain information on the plants used by humans (freelisting) might be adequate for exploratory research but it might not be the optimal approach for comparative studies. Information provided during the interviews, relies on people's memory and answers could be influenced by external factors such as time/season when the interview was conducted and what action the person was performing at the time (Chaves et al. 2019). For example, in Hockings and Sousa (2013), *Treculia africana* was reported as having a symbolic and religious meaning to people of Guinea-Bissau, fruit being eaten by children and wood used in construction. Despite this tree being present in Cantanhez NP and detected in the diet of red colobus and chimps, it was never mentioned in the interviews.

At the biological level, a number of factors could have influenced the quality and quantity of plant DNA extracted and sequenced with DNA metabarcoding, creating a bias mainly towards chimpanzee diet: Sample collection – chimpanzee faecal samples are larger than red colobus pellet, hence only a small portion of the whole faecal matter detected in the field, was collected. Multiple sampling replicates would probably have captured a higher plant diversity consumed by the individual but this strategy was more time demanding, prone to contamination and costly so it was not implemented (Mata et al. 2019; Ando et al. 2020); Sample storage – the samples were desiccated in silica gel rather than a 2-step method, which involves submerging the samples in ethanol for 24-48 hours and later desiccate it in silica. Unfortunately, again for chimpanzee samples, full desiccation was not complete for some samples and fungal growth occurred, possibly reducing DNA yield during DNA extraction and inhibiting DNA PCR amplification. Later in the high-throughput sequencing step, the universal plant marker ITS2 could have amplifying fungi over plant DNA (Schoch et al. 2012); Plant DNA extraction - some chimpanzee samples contained many seeds and little faecal matter, which could have reduced the initial sample yield that provided higher quantities of DNA. One other cause for this result could be digestion bias, but more research should be performed in this field to gain insight into digestion, retention and processing times, and nutrient /plant DNA accumulation in faeces; Plant taxonomic identification – although ITS2 reference database has increased in the past years, many plants such as West African species, have not been sequenced for this marker or ribosomal DNA. This was the case for *Dialium guineense*, which was clearly present in the faeces of the chimpanzee (seeds identified in the DNA extraction step) but not detected in the DNA metabarcoding due to the lack of sequences in the reference database. In addition, even though chimpanzees are fruit specialists, individuals are known to complement their diet with non-plant items (Pruetz 2006). Hence, if another marker suitable for the amplification of vertebrate and invertebrate DNA (e.g. mitochondrial cytochrome oxidase I) would have been used, a broader range of food items of the omnivorous chimpanzees would have been revealed. This addition would have allowed for a more thorough assessment of complementary items consumed at times of fruit scarcity.

5.5.5 Conservation importance

Overlap rates of wild plant use between humans and the two primates were observed in Gola RNP and Cantanhez NP, and some plant taxa were identified as important for all or across some groups. Furthermore, cross-area and species comparative studies provide a broader view of the use of the forest and its resources, but also more species and site specific information that can be passed on to conservation management teams in order to develop or improve programs of forest conservation and reforestation. Due to the wider and more focused information obtained, these programs can address widely distributed plant species, or specific areas and plants in the parks which have been identified as playing a crucial role in the survival of the endangered primates, and wellbeing of humans. Though variation in co-use of wild plants is common, data on valuable natural resources overlap should be incorporated into evidence-based conservation strategies and policies (IUCN SSC 2020; Linder et al. 2021).

The genus *Ficus* is a good example of a widely distributed plant taxon that is integral of most primate diets, and is used for multiple purposes across human communities.

This plant can produce fruit and leaves nearly all year round, and has the ability to populate various habitat types (e.g. forest, savanna, wetlands, etc). Even though most species are not threatened, their population trend is decreasing. This taxon has been frequently detected in both primate species of this study living in a natural (Goal RNP) and a disturbed habitat (Cantanhez NP), and across many other primate feeding ecology studies. For orang-utans in Indonesia, figs were reported as critical for releasing populations from the typical pressures of an intermittently low food supply in a natural Sumatran forest (Wich et al. 2006). In the fragmented patches of gallery forest along the banks of the lower Tana River, Kenya, this fruit dominated the diet of the red colobus monkey *P. kirkii* (Struhsaker 2010). For people in West Africa, *Ficus* trees, shrubs and lianas products also represent a source of food for themselves and cattle, fuel for cooking, natural medicine, and have a culture importance for some (e.g. cloth and raffia dyeing, use the sap as glue in traditional hunting tools) (*PROTA4U* 2022). In sum, conservation of commonly utilised taxa, should be prioritised in monitoring and forest conservation species and habitat plans to minimise the impact and guarantee security for endangered primate species, and contribute to the preservation of human livelihoods and culture.

Fine scale investigations could identify endemic plants which might be frequently consumed by regional threatened primate, and that contribute specifically to the lives of local human communities (Hockings et al. 2020), or reveal complex dynamics between wildlife and humans on the use of dietary and economically important wild plants (Ramon 2017). In the continuous well preserved moist deciduous forest of Gola RNP, for example, a recent report has identified areas of high risk of deforestation which are home and feeding grounds, identified in this study, to species of threatened primates (RSPB 2021). In a model of land cover for 2028, the area in Gola South sampled during this research is completely transformed into non-forest area, and part of the are surveyed in Gola Central will likely experience the same levels of land transformation. Given such predictions, it is crucial to cross and integrate information obtained in this and other studies (e.g. hunting pressure and primate behaviour - Foglietti 2020; chimpanzee nest ecology and conservation - Barca et al. 2018a) to best inform stakeholders of particular species needs, to take directed decisions and create measures that can be applicable and successful. Furthermore, *P. b. badius* fed on 12 plant species which are considered near threatened (e.g. *Didelotia idea*), vulnerable (e.g. *Lophira alata*) or endangered (*Dactyladenia smeathmannii*). Only one species had a salience index for humans in this study, *Garcinia afzelii*, but other plants in the threatened list were previously reported as being used by people (e.g. Nauclea spp., Fleroya spp., Daniellia spp.) (Munro et al. 2013; Jusu and Sanchez 2014). As monkeys and apes are considered aiders in seed dispersal along with elephants and ungulates, also present in the park, further research in this field could result in strategies for community awareness of the importance of protecting and conserving primates, and other wildlife, for the maintenance of a sustainable forest, including populations of plant species important for people.

In the case of the already disturbed and fragmented forest of Cantanhez NP, the situation cannot be reversed, but it can be prevented from progressing to worst case scenarios. Considering the relative stability of vegetation cover over the past seven decades (Catarino and Palminha 2018), the high amount of threatened species in the park, and the considerable number of multidisciplinary and species studies developed in the area, this NP could be a case study for evidence-based conservation of one of the most common and complex systems globally, the agroecosystem (Estrada et al. 2012). This research has demonstrated that levels of wild plant overlap between sympatric humans and two primate species was high, and allowed for the identification of six important plants, with varying degrees of salience in primates diet and peoples' use. Most of these plants are not the most common across the park (e.g. *Phyllanthus muellerianus*) or are restricted to a restricted number of habitats (e.g. *Anisophyllea laurina*), and if unsustainable exploitation takes place, primate populations may be compromised and threatened to extinction.

Even though results from this research support the idea that both primates have some degree of flexibility to changing environments, this is only related to the capacity to alter their diet and not to survive habitat alterations. However, these adaptations can only be carried out if there is enough forest left to provide the nutritional requirements for the species (Nowak and Lee 2013). Furthermore, the cost of living in degraded habitats involves many other facets of the species' biology and ecology (e.g. traveling longer distances in search of food, higher exposure to predators), and if their habitat is devastated in quantity and quality, these animals will most likely face extinction (Estrada et al. 2017). With this study, I do not wish to diminish the necessity to protect primate natural habitat. In fact, this study is aimed at gaining a detailed knowledge on the specific dietary requirements of each primate species, and how they share wild plants with local human population, to promote the impact these wild animals have on the ecosystem maintenance and consequently human wellbeing, in the hope that primates and forests will be considered in local and wider conservation programmes.

One additional thought to consider when disseminating this dietary data and plant use information to stakeholders, is that the plant overlap investigated in this research refers only to the plants primates fed on, but these animals use these and other plants for locomotion, as sleeping sites, and even as tools for feeding. Nonetheless, the aim of this study was to identify plants consumed by primates that are also important for people.

Ultimately, understanding the social-ecological system is important to achieve a successful conservation strategy balanced between guarding biodiversity and allowing people's access to natural resources in a sustainable way. National and regional resource use regulations should be designed with the absolute inclusion of the direct users of such resources and experts which hold detailed information on the species ecology.

CHAPTER 6. General Discussion

Velvet tamarind (*Dialium guineense*) seeds fed by Temminck's red colobus (*Pilicolobus badius temminckii*) in Cantanhez National Park, Guinea-Bissau.

6.1 Review

This dissertation has evaluated the extent of wild plant overlap between sympatric humans and non-human primates in two NPs in west Africa. To perform a comparison of wild plant use between the two groups, I used an integrative approach that combined social and biological methodologies. Data were concurrently collected on the plants contributing to the diet of the chimpanzee (*Pan troglodytes verus*) and the western red colobus (*Piliocolobus badius*), and on the wild plants used by human communities in and around each NP. The two study sites selected were a continuous moist evergreen forest in southeast Sierra Leone (Gola Rainforest National Park) with no settlements and low levels of human activity within the protected area; and a mosaic of sub-humid and secondary forest, mangrove, savanna, and agricultural fields in the south of Guinea-Bissau (Cantanhez National Park) with 110 villages in the protected area and high levels of forest disturbance. I assessed the dietary flexibility of the two primates in the disturbed habitat in comparison with the 'natural' habitat and tested if the degraded habitat drove co-existing humans and primates to increase wild resource overlap. A picture of each primate's dietary diversity and the overlap between sympatric primates emerged, along with a list of the most salient trees and plants to human communities in parks with different management characteristics. Levels of co-use of wild plants by both groups were calculated, as well as cultivated foods that might potentially be an alternative food to primates in times of natural food shortage. To date, this is the first study that uses a cross-disciplinary approach to perform a multi-species analysis on wild plant co-use at a finer and broader geographical scale. This is also the first time that a DNA-based method has been used to conduct a dietary characterisation of one ape and two western red colobus subspecies. With this research, I hoped to contribute to the conservation of a critically endangered ape and endangered colobines in two protected areas supporting some of the most important populations of these primates. Lastly, by providing a better understanding on resource co-use, I aimed to contribute to the planning and implementation of an evidence-based conservation approach in both NPs.

6.2 Overview of main findings

6.2.1 Chapter 2. Wild plants as a natural resource for human communities living within and outside West African national parks.

The results obtained through structured oral interviews conducted with people living in villages near or inside protected areas, revealed high levels of wild plant use in both study sites. This result was expected considering the strong dependence on nature reported worldwide, and particularly in the African continent where nearly half of the population rely on natural resources for their basic needs. However, the hypothesis proposing that wealth and increased distance from the village to the mature forest in Gola RNP, Sierra Leone, would discourage people from harvesting wild plants was not supported by the results. The high levels of wild plant use observed and the dissociation between demography and geography is an indicator that most people are still very dependent on wild plants to provide first care assistance. This result falls in line with the poor health care available in Sierra Leone, in particular rural areas; shortage of health clinics in rural areas and the prohibitive cost of standard health services for the majority of the population, in a country that has one of the world's poorest per capita incomes (World Bank 2020). Moreover, many NTFPs are sold in markets, demonstrating the cultural and economic importance of natural resources to urban and rural people. However, sustainable extraction should be the aim in projects developed for forest protection, and this should involve the parties that directly benefit from NTFPs and carry local knowledge, which is sometimes not achieved with unidimensional ecological research approaches.

Variation in wild plant use was thought to be influenced by demographic variables, as societies in both study sites are patriarchal and hierarchical, yielding differences in the characteristics and capabilities of each gender and social age group (Leach 1994). However, only in Cantanhez NP did demographic factors explain differences in the number of plants listed by different groups of people. In general, elders and people with Koranic education listed higher numbers of wild plants than other groups. As expected, variation between community groups was observed, indicating differences in people's relationship with the natural environment. A comparison of the level of wild plant use by humans between the two study sites, showed that people in Cantanhez NP, Guinea-Bissau, who are immersed in the protected habitat, demonstrated a higher knowledge of wild plants than villagers living on the outskirts of a protected forest heavily managed for conservation (Gola RNP in Sierra Leone). However, these differences were not significant.

The top ten most important plants for communities in both NP were trees, shrubs and climbers, which were used for various basic needs, such as traditional medicine, construction, food and fuel. Both parks' vegetation is characterised as sub-humid forest comprising large trees such as *Ceiba pentandra* and *Parinari excelsa* (Scott 1992; Rodrigues et al. unknown). People listed an average of 5 and 8 plants per informant, in Gola RNP and Cantanhez NP, respectively. Thirteen of the species were common across parks, and four had similar salience scores: *Anisophyllea laurina*, *Cassia sieberiana*, *Sarcocephalus latifolius* and *Sterculia tragacantha*. Some trees are found in the dense primary forest while others are present in forest regrowth areas, suggesting that people use different types of habitat to harvest specific trees. Although this research suffers from some limitations, I argue that the methodology applied in this chapter is a good approach for an exploratory study that can provide a broad view of the factors influencing people's use of wild plants, can identify plant species important for local human communities, and provide specific evidence for the development of reforestation plans.

6.2.2 Chapter 3. DNA metabarcoding: a new insight into the diet of the critically endangered western chimpanzee (*Pan troglodytes verus*).

The diet of western chimpanzees was characterised and evaluated in this chapter, using a DNA metabarcoding approach applied to non-invasive samples. This method was chosen as the study species are unhabituated and elusive primates, inhabiting a dense continuous forest in Gola RNP, Sierra Leone, where detection of individuals is difficult due to visibility and extension of habitat; and another population inhabiting the anthropogenic fragmented forest of Cantanhez NP. The low number of samples obtained in Gola RNP, also reflect the low density of these animals in the park. Nevertheless, the first time a DNA-based method was applied to study the diet of an ape, has proven to be successful at detecting plant species in the diet of the Critically Endangered *Pan troglodytes verus* at both sites, including plant species rarely eaten by chimpanzees. Dietary diversity was high for both populations, despite the low number of samples in Gola RNP. In comparison with other studies that used traditional methods to perform faeces examinations, the total number of plants detected was lower but I believe this is due to the smaller number of samples and shorter sampling period of the current study. Chimpanzees fed on plants commonly found in dense primary forests, but also on species present in areas of forest regrowth. This result in Gola RNP is probably explained by the high number of samples collected in the community forest, which is typically a secondary forest with cultivated fields. This also could explain the high presence of one crop (*Abelmoschus* spp., 11%) in the population diet. Results suggest that chimpanzees are foraging more often outside the protected forest, but a more exhaustive sampling in Gola RNP is required to understand if chimpanzees have a preference for anthropogenic habitat or if the characteristics of work developed in a dense forest influenced the results in this study.

The average number of plant taxa included in the diet of each chimpanzee was lower in Cantanhez NP than in Gola RNP, possibly indicating a poorer quality of the habitat, as expected in anthropogenically environment. However, the population revealed a high dietary flexibility in the disturbed habitat by feeding more frequently on two important plants present across most habitat types in the park (*Ficus* spp. and *Ceiba pentandra*) and by integrating a high percentage (12.5%) of cultivated items in their diet. Crop consumption is similar to data obtained for other populations living in other sites with high exposure to farms. Diet composition differed significantly between the dry and rainy seasons with individuals consuming more plant species in the dry season as a result of ripe fruit availability in the park. Plants consumed in the rainy season have been recorded in other studies and its plant parts were other than ripe fruit, indicating that chimpanzees feed on other plant parts when ripe fruit availability is low. Chimpanzees in Cantanhez NP also demonstrated a preference for some crop species, as mango and cashew fruits were exclusively consumed in the dry season when wild ripe fruit is highly available. Overall, the population of chimpanzees in Cantanhez NP appears to have adapted their diet to the type and quantity of wild food resources available in the park, but the high presence of crops in their diet indicates the possibility of encounters with people upon crop feeding events and when travelling between forest fragments, putting the population at risk. Research and work with local people needs to be continued to address the many aspects of the complex social-ecological system these chimpanzees live on.

6.2.3 Chapter 4. Ecological and temporal variation in the diet of the two western red colobus (*Piliocolobus badius*) subspecies

The data used in this chapter were obtained through the collection of non-invasive samples from non-habituated populations of western red colobus inhabiting two distinct protected areas in West Africa. The Bay colobus (*Piliocolobus badius badius*) is one of five largest populations of this species with circa 5,000 individuals (McGraw et al. 2020). Occupying the continuous protected area of Gola Rainforest National Park in southern Sierra Leone, this population is threatened mainly by deforestation and hunting. Many populations of red colobus are understudied (Linder et al. 2021), and efforts to understand better the Bay colobus of Gola RNP are being made. Currently, population conservation genetics research is being conducted in this group of primates. And this study, has contributed with the first high resolution description on this population’s diet. A DNA-based metabarcoding approach revealed high levels of dietary diversity sustained by Bay colobus suggesting that Gola forest provides the required wild plants essential for the red colobus diet. However, evidence of low levels of crop consumption was detected, indicating that the primates may be experiencing some decrease in preferred food items available in the forest complementing it with cultivated items. However, this behaviour could also be a result of food preference, as crops were most frequently ingested in the dry season when many plants bear young leaves or are fruiting. The crop consumed at a higher frequency was *Coffea* spp., a plant that is grown in an agroforest system, under the shade of high canopy trees. This habitat could be suitable for red colobus and the unripe fruits of *Coffea* spp. attractive as a high-quality food item.

The other population studied was the Temminck's red colobus (*Piliocolobus badius temminckii*), found in the fragmented and anthropogenically impacted forest of Catanhez National Park in Guinea-Bissau. This Endangered species represents a large but declining population of this subspecies due to being heavily threatened by habitat reduction and hunting (Minhós et al. 2020). The same methodology was used to produce the first dietary study on this sub-species in Guinea-Bissau. Temminck's individuals also showed a preference for plant species present in primary forest (e.g. *Treculia africana*), but their diet also included species characteristic of other habitat types found at Cantanhez NP, such as secondary forest growth areas. In comparison with Gola RNP population, *P. b. temminckii* had a lower dietary diversity, but crops represented a much higher proportion of the plants in their diet (11%), with a high preference for mango tree. These results suggest some level of dietary adaptation to possibly lower levels of wild food availability presented in the disturbed forest of Cantanhez. Temporal differences were assessed with a 10 year gap but no significant differences were detected at the species richness level. Although nearly 50% dietary overlap was observed between individuals at the two time points, the remaining plants were only fed by individuals in one of the years. Abundance or phenology data would have contributed to a better understanding of this variation in choice of plants along time, as it is most likely related to quality and food availability in the forest fragments inhabited by red colobus groups.

6.2.4 Chapter 5. Wild plant overlap between sympatric humans and non-human primates in a continuous rainforest and an anthropogenic landscape.

Social and biological data were collected to develop this research, and in this chapter I integrate the two disciplines to understand and explain some of the patterns observed in protected areas where humans and non-human primates share space and resources. The results from chapter two provided a broad perspective of wild plant use by local human communities living within or outside protected areas with similar policies of forest use. In Gola RNP people must travel to reach the mature protected forest and their villages, though outside the park, are established in a community forest that in most places resembles a mature forest. In Cantanhez NP, people live among the forest fragments of the NP, farming and maintaining their orchards closer to the settlements. Lists of the most important wild plants for each community were obtained using a freelisting methodology. In both NPs, the use of NTFPs is high demonstrating the dependence of people on the forest and its products.

In chapters three and four, I obtained a detailed description of the diets of the Critically Endangered western chimpanzee and the Endangered western red colobus. Through the implementation of a DNA metabarcoding approach, it was possible to identify the plants contributing the most to each primate diet, and some preferred food items. The data further allowed for the detection of cultivated plants consumed by the two non-human primates in each NP. Both primates sustained high levels of plant richness in their diets, with chimpanzees demonstrating a narrower dietary niche than red colobus at both study sites, as expected for a frugivorous primate. Possibly as a response to food resources quality reduction in the disturbed Cantanhez forest, both primates had a wider dietary niche, but this could also be a result of higher intra-species variation in the diet reflecting the more diverse landscape observed in Cantanhez NP than in Gola RNP. Dietary niche overlap between sympatric primates was in the order of 0.3, but a higher overlap was observed in Cantanhez NP than in Gola RNP. I assume that this increase in co-use of food species is due to the need for chimpanzees and red colobus to share more wild resources at times when their preferred food availability is limited.

Ultimately, the combination of results from different fields of work permitted an assessment of the co-utilisation of wild plants by humans and non-human primates. The levels of overlap were nearly twice as high in Cantanhez NP than in Gola RNP possibly due to the humans living within the protected area. This ratio increased when crops where included in the overlap measure, most likely because primates have easier access to cultivated areas in the disturbed habitat. Unexpectedly, red colobus shared more resources with humans than chimpanzees. This could be an effect of sample size (higher for red colobus than for chimpanzees), a result of better quality samples for red colobus providing higher resolution of species diversity than in chimpanzee samples, or simply an effect of the smaller number of studies on human/non-human primate interactions for colobines than for chimpanzees. Information resultant from this chapter allowed for the identification of some important plant species for humans and non-human primates, which could be used in evidence-based conservation plans for habitat regeneration programmes and primate conservation strategies.

6.3 Conservation implications

The main aim of this project was to produce new insights on the co-use of wild plants by sympatric human and non-human primates in two NPs with distinct conservation characteristics. By using social methods, I could determine the type of plants which are most important to people residing within and around the protected forests. When using a DNA-based technique to explore the diet of two threatened primates, I was able to obtain a detailed list of the plants consumed, including which were more important or preferred among primate populations, and how well primates adapt their diet to a more disturbed habitat. The combination of the two disciplines, allowed for an overview of how much resource overlap is currently existing in each park, and the recognition of specific plants that might be crucial to humans and non-human primates, and assess the benefits or possible conflicts of co-utilisation.

Gola RNP not only provides natural land and resources to local communities, as it offers critical ecosystem services such as watershed protection, erosion prevention and climate regulation, and most importantly carbon sequestration (RSPB 2021). Currently, the protected area features a healthy and large continuous forest, but predictions suggest high levels of deforestation in the next six years, especially in the protected forest edge areas (RSPB 2021). This is a real risk for local flora and fauna, especially for the critically endangered chimpanzee and the endangered Bay colobus. This park supports some of the largest populations of both species, roughly estimated at 5,000 individuals per taxon. With shrinking habitat and anthropogenic expansion, these animals will be more exposed to survival threats, such as hunting, conflicts with humans, and disease (Goldberg et al. 2008; Estrada et al. 2012). Just as importantly, an impoverishment and reduction in food resources will compromise the viability of these primate populations.

At the same time, Gola residents will see their natural resources decrease in availability, forcing them to continue to travel deeper into the forest to obtain important wild plants. As habitat quality declines, primates will probably search for energy-rich and easily digestible food alternatives in cultivated areas, as has been observed in Cantanhez NP. This behaviour increases the chances of human-primate conflict, and it may have an impact on the economy and food security of human populations which are already experiencing very high levels of extreme poverty (World Bank 2020). Consequently, reduced access to protein rich food in human diets may increase the need for primate meat hunting.

The near future scenario for Gola RNP is not promising, but since we do know the factors that may lead to such impactful results on the social-ecological ecosystem, conservation efforts need to be rapidly developed and implemented. One solution is reforestation and mitigation actions against deforestation, but these programmes need to be well-planned to avoid problems that may arise from rapid strategies that are not well thought through. For examples, the type of tree and habitat where the trees are planted is crucial for a sustainable ecosystem maintenance, for the economic equality in the region, and for the avoidance of land dispossession (see examples in Holl and Brancalion 2020). Sometimes, cash crops are a proposed solution and implemented by governments to promote the local economy and forest regeneration, but even though forest cover increases, it does not respond to the other problems associated with deforestation, such as biodiversity loss. Guinea-Bissau, including Cantanhez NP, has an example of a rapid solution to deforestation that was not best planned. Temudo (2009) explains how the cashew tree (*Anaccardium occidentale*) was initially used as a governmental measure to improve the local economy, and later implemented in a national reforestation programme. Although forest cover increased or has been maintained in some areas, as is the case of Cantanhez NP (Catarino and Palminha 2018), this monoculture has not benefitted biodiversity in any measurable way.

Due to the lack of phenology data in this study, it is difficult to identify possible fallback foods for chimpanzees and red colobus (available year-round but usually consumed of low preferred food availability) (Marshall et al. 2009). However, *Ficus* spp. has been previously identified as a fallback food for this population of *P. t. verus* (Bessa et al. 2015), and this was the taxa most present in chimpanzee diet in this study in Cantanhez NP. Exemplars of this genus could be considered in reforestation programs, along with other important plants for primates and people, and people only. Large trees such as the Guinea pepper (*Xylopia aethiopica*), African crabwood (*Carapa procera*), Monkey apple tree (*Anisophyllean laurina*) and rough-skinned plum (*Parinari excelsa*) are already being planted by some community members interviewed. These respondents, together with people who listed a large number of wild plants in our interviews, could be proposed as ambassadors of reforestation programs. Besides having knowledge on the type of plants important to the community, their uses, as well as practical information on how to cultivate the plants, such ambassadors can better communicate to the rest of the population, as they are part of the group that directly depend on NTFPs, and demonstrate that this action is easy and feasible to achieve.

Reforestation, particularly in Cantanhez, would provide forest corridors for primates to travel safely between the small fragments of dense forest to find food, shelter and improve their choices of food items. If more trees offering the type and quantity of foods these primates prefer were found in forests which are further away from the settlements, then primates would not need to travel in search of food and increase the chances of human-primate encounters. Red colobus would probably reduce their crop feeding, if large trees like *Treculia africana* and *Parinari excelsa* were more available in the dense forest. This may not be the case for chimpanzees, as in the park they feed on cashew and mango, and other crops even during the fruiting season (Bessa 2014, and current study). In this case, alternative solutions to deter chimpanzee crop feeding should be examined. In Kibale NP, Uganda, for example, agroforestry buffers including key forest fruit like *Mimusops bagshawei*, have been proposed (Naughton-Treves et al. 1998). Apart from reforestation plans, strategies to minimise or impede further forest loss should be implemented. The preservation of important trees for frugivorous and folivorous primates could be preserved through selective felling (Chapman and Chapman 2002), and by controlling the intensity and frequency of harvesting, as well as avoiding the plant parts critical for the long-term survival of the species (Belcher and Schreckenberg 2007; Hernández-Barrios et al. 2015).

Both NPs propose management strategies that must involve governmental agencies, NGOs, Universities and local communities, and integrate various disciplines. Ethical considerations are particularly important in regions where land tenure is culturally complex, to avoid conflict between conservationists and local communities. Although research is commonly conducted in both NPs, there is still a large knowledge gap on how sympatric human and primates interact and cooperate in complex ecosystems. Specifically, wild plant overlap and crop feeding by primates could be further explored to understand which strategies could be implemented to benefit both threatened primate populations and human livelihoods. Moreover, I believe that further investigation should be conducted on the benefits and costs of having large community forests around settlements and NPs devoid of human settlements. Such areas could reduce human impact on primary forest and could also create a buffer for wildlife as a way to protect human cultivated foods.

6.4 Conclusion and future development

With expanding human populations and transformation of land into agricultural and urbanised areas, primates are increasingly having to inhabit areas that are in close proximity to humans and adapt to these new environments (Estrada et al. 2012; Kalbitzer and Chapman 2018). Therefore, it is crucial for contemporary conservation, to understand the mechanisms behind human and non-human primate co-existence. One area of interest is the sharing of natural resources, as all groups use wild plants for food, tools, and shelter, among other uses for humans. Studies conducted at a fine spatial scale are important to disentangle the complex interaction between the environment, anthropogenic factors and species biology and ecology, but comparative studies like this one, can also provide a broader view of how primates might change their dietary behaviour and adapt to modified landscapes, including interacting with humans. Protected areas prioritise ecosystem and biodiversity conservation, alongside protecting local people and cultures. Strategies preferred would be engagement into co-management and development initiatives, recognising the right of use within people’s territory and incorporating sustainable use of natural resources (Beltran and Phillips 2000). Harvesting of NTFPs can be presented as an aid for the conservation of biodiversity and development of rural communities on a sustainable basis (Godoy and Bawa 1993; De Jong et al. 2000), rather than being ignored.

This study provides key information not previously available, that can be applied in evidence-based conservation. However, further research could complement and better explain some of my results. Phenology and abundance data on the plant species in the parks would allow a deeper understanding of some of the co-use patterns observed, and to use null models exploring niche overlap between the two primates, and extend it to overlap with the human populations (e.g. econullnetR package, Vaughan et al. 2018). Due to the nature of DNA metabarcoding, data has been produced for many other primate species collected during field work. By incorporating dietary data from other sympatric monkeys into the current results, I could obtain a broader picture of the co-use of wild and cultivated plants in each natural ecosystem of Gola RNP and the agroecosystem in Cantanhez NP. These results would once more be combined with social data to evaluate species flexibility to disturbed habitats and interactions with people. These primates also have different degrees of forest use, such as the King colobus (*Colobus polykomos*) are more susceptible to forest changes as with the western red colobus, but others are more likely to use disturbed habitats as is the case of the spot-nosed monkey (*Cercopithecus petaurista*) which was frequently observed near villages and crops in Gola RNP (pers. obs.). Most primates are omnivorous and many are insectivorous, with chimpanzees also feeding on small mammals and other monkeys. So, by sequencing the same samples with a specific marker to detect invertebrates and vertebrates (e.g. the mitochondrial cytochrome oxidase I gene) it would be possible to have a complete description of the diet of the primates of Gola RNP and Cantanhez NP. DNA metabarcoding is a technique that benefits from the addition of other molecular markers to obtain a better resolution on taxa detected in non-invasive samples. In this case, samples could be sequenced using a chloroplast marker (e.g. *trn*L) to identify some taxa that did not have enough sequence resolution to be identified to the species level (e.g. *Abelmoschus* spp. of *Ficus* spp.). Furthermore, improving the reference genome NCBI GenBank database for West African plants would provide a better resolution of taxonomic identification. Creating a local database was initially planned with Dr. Lebbie and a PhD student from Njala University, Sierra Leone, but had to be left out due to funding constraints.

Lastly, as a result of a pandemic most likely originating from a zoonotic disease, a high number of studies have been published looking at the human-wildlife interaction from a co-existence point of view rather than a conflict approach. Although I tried to integrate some of the research in this project, most studies were produced at a later phase of this thesis writing and were not included in the literature research nor extensively in the discussion. Yet, I hope to integrate some of the newer results in my future work and scientific publications, as I feel the research produced in this PhD can contributed to expand knowledge about human-wildlife co-operation and co-existence, and the mechanisms behind such a complex social-ecological system.

Appendix 1 – Supporting material for CHAPTER 1

Table A.1.1 Types of vegetation found in Gola RNP

Habitat location and most common plants recorded for each habitat. Life form, plant family and global conservation status is presented for each plant species. Species in bold are the most abundant across the NP.

Habitat type	Location most commonly found at	Main plant species	Life form	Plant family	Status
Evergreen forest	Gola Central	***Heritiera utilis***	T	Malvaceae	VU
		Brachystegia leonensis	T	Fabaceae	VU
		Calpocalyx aubrevillei	T	Fabacecae	LC
		Sacoglottis gabonensis	T	Humiriaceae	LC
		Uapaca guineensis	T	Phyllantaceae	LC
		Protomegabaria stapfiana	T	Phyllantaceae	LC
Moist semi-deciduous forest	Gola South	***Cynometra leonensis***	T	Fabaceae	NT
		Parinari excelsa	T	Chrysobalanaceae	LC
		Parkia bicolor	T	Fabaceae	LC
		Piptadeniastrum africanum	T	Fabaceae	LC
		Erythrophleum ivorense	T	Fabaceae	LC
		Nesogordonia papaverifera	T	Malvaceae	VU
Freshwater inland swamp forest	Evergreen and semi-deciduous forest	Raphia palms	T	Arecaceae	LC, NT
		Uapaca spp.	T	Phyllantaceae	LC
		Nauclea diderrichii	T	Rubiaceae	NT
		Newtonia duparquetiana	T	Fabaceae	LC
		Carapa procera	T	Meliaceae	LC

Forest regrowth and secondary forest	Old logged areas in Gola South, and western part of Gola Central	*Macaranga barteri*	T	Euphorbiaceae	LC
		Musanga cecropioides	T	Urticaceae	LC
Farmbush, herbaceous swamps and floodplains	In and around the protected forest	*Chromolaena odorata*	T	Asteraceae	LC
		Acacia spp.	S, T	Fabaceae	LC
		Adenopodia spp.	S	Fabaceae	VU, EN
		Herbaceous vegetation	-	-	-

All forest types can be found in the community forest. Life form – tree (T), shrub (S). Status - IUCN Red List Conservation status: LC (least concerned), NT (near threatened), VU (vulnerable), EN (endangered). Source: Klop et al. (2008), IUCN (2022).

Table A.1.2 Types of vegetation found in Cantanhez NP

Habitat location and most common plant species recorded for each habitat. The life form, plant family and global conservation status is presented for each plant species. Species in bold are the most abundant across the NP.

Habitat type	Most commonly found in	Main plant species	Life form	Plant family	Status
Primary forest (Dry forest)	Isolated forest patches. It provides NTFPs such as fruit, palm oil, wood, honey, wood for fire and charcoal	*Albizia dinklagei*	T	Fabaceae	LC
		Alstonia congensis	T	Apocynaceae	LC
		Anisophyllea laurina	T	Anisophylleaceae	LC
		Dialium guineense	T	Fabaceae	LC
		Elaeis guineensis	T	Aracaceae	LC
		Hunteria umbellata	T	Apocynaceae	LC
		Klainedoxa gabonensis	T	Irvingiaceae	LC
		Malacantha alnifolia	S, T	Spotaceae	NA
		Strombosia pustulata	T	Olacaceae	LC
		Parinari excelsa	T	Chrysobalanaceae	LC
Secondary forest (forest regrowth)	Old dense forest degraded as a result of anthropogenic pressure. Frequently, fruit trees are planted in these areas.	*Afzelia africana*	T	Fabaceae	VU
		Albizia adiantifolia	T	Fabaceae	LC
		Alstonia congensis	T	Apocynaceae	LC
		Antiaris toxicaria	T	Moraceae	LC
		Cassia sieberiana	T	Fabaceae	LC
		Ceiba pentandra	T	Bombacaceae	LC
		Detarium senegalense	T	Fabaceae	LC
		Dialium guineense	T	Fabaceae	LC
		Elaeis guineensis	T	Aracaceae	LC

		Ficus sur	T	Moraceae	LC
		Malacantha alnifolia	S, T	Sapotaceae	NA
		Markhamia tomentosa	S, T	Bignoniaceae	LC
		Parinari excelsa	T	Chrysobalanaceae	LC
		Spondias mombin	T, S	Anacardiaceae	LC
Savannah woodland	Rich in herbaceous plants, resultant from natural or human induced fires	*Albizia zygia*	T	Fabaceae	LC
		Bombax costatum	T	Malvaceae	LC
		Borassus aethiopum	T	Arecaceae	LC
		Bridelia micrantha	T	Phyllanthaceae	LC
		Combretum nigricans	T	Combretaceae	
		Daniellia oliveri	T	Fabaceae	
		Elaeis guineensis	T	Aracaceae	LC
		Ficus glumosa	T	Moraceae	LC
		Khaya senegalenesis	T	Combretaceae	LC
		Lannea velutina	T	Anacardiaceae	LC
		Parkia biglobosa	T	Fabaceae	LC
		Pterocarpus erinaceus	T	Fabaceae	EN
		Aframomum spectrum	H	Zingiberaceae	LC
		Ipomoea eriocarpa	H	Convolvulaceae	LC
		Vigna gracilis	H	Fabaceae	LC
		among others		Fabaceae	
Palm groves	Flat areas and/or with a slight slope, usually bordering mangroves or ‘lala’ (floodplain/lowland). High human presence for oil palm extraction mainly.	*Elaeis guineensis*	T	Aracaceae	LC
		Albizia spp.	T	Fabaceae	LC
		Anthocleista vogelli	T	Gentianaceae	LC
		Carapa procera	T	Meliaceae	LC

		Cassia sieberiana	T	Fabaceae	
		Dialium guineense	T	Fabaceae	LC
		Ficus ovata & sur	T, S	Moraceae	LC
		Khaya senegalensis	T	Meliaceae	VU
		Malacantha alnifolia	S, T	Sapotaceae	NA
		Milicia regia	T	Tree	VU
		among others			
Riparian forest	Gallery forests near freshwater bodies.	*Anthocleista procera*	T	Gentianaceae	LC
		Anthostema senegalense	T	Euphorbiaceae	LC
		Elaeis guineensis	T	Aracaceae	LC
		Ficus lutea	S, T	Moraceae	LC
		Hymenocardia heudeloti	S, T	Phyllanthaceae	LC
		Mitragyna inermis	S, T	Rubiaceae	LC
		Myrianthus serratus	S, T	Urticaceae	LC
		Pterocarpus santalinoides	T	Fabaceae	LC
		Uapaca guineensis	T	Phyllantaceae	LC
		among others			
Mangroves	Coastal areas (low species richness).	*Avicennia germinans*	T, S	Avicenniaceae	LC
		Rhizophora spp.	T, S	Rhizophoraceae	LC
		Conocarpus erectus	S	Combretaceae	LC
		Laguncularia racemosa	S	Combretaceae	LC
Herbaceous savannah	‘lala’, in floodplains and lowland, markedly seasonal (floods in the rainy season and dries completely in the dry season).	*Anadelphia afzeliana*	H	Poaceae	NA
		Ipomoea septifera	H	Convolvulacee	NA
		Vigna gracilis	H	Fabaceae	LC
		Elaeis guineensis	T	Aracaceae	LC

		Mitragyna inermis	S, T	Rubiaceae	LC
		Nauclea latifolia	T	Rubiaceae	LC
		among others			
Fallows	Mixed plantations, present in the region for many decades. Some natural vegetation co-occurs.	*Cola nitida*	T	Malvaceae	LC
		Mangifera indica	T	Anacardiaceae	DD
		Alstonia congensis	T	Apocynaceae	LC
		Dialium guineense	T	Fabaceae	LC
		Elaeis guineensis	T	Aracaceae	LC
		Parinari excelsa	T	Chrysobalanaceae	LC
		Musa spp.	H	Musaceae	LC
		among others			
Cropland	Monoculture. Natural vegetation absent. Although common, these plantations are relatively small, rarely being over one hectare.	*Anacardium occidentale*	T	Anacardiaceae	LC
Orchards	Recent plantations. Due to the monoculture method, the soil in these orchards becomes infertile, contrary to what happens in traditional orchards.	*Citrus* spp.	T, S	Rutaceae	LC
		Mangifera indica	T	Anacardiaceae	DD
		Musa spp.	H	Musaceae	LC
Other orchards	Near settlements and roads	*Several crops*			

Life form – tree (T), shrub (S), herb (H). Status - IUCN Red List Conservation status: NA (not available), DD (data deficient), LC (least concern), VU (vulnerable), EN (endangered). Even though most species conservation status is least concern, their global population trend is decreasing. Source: Temudo (1998); Catarino and Palminha (2018).

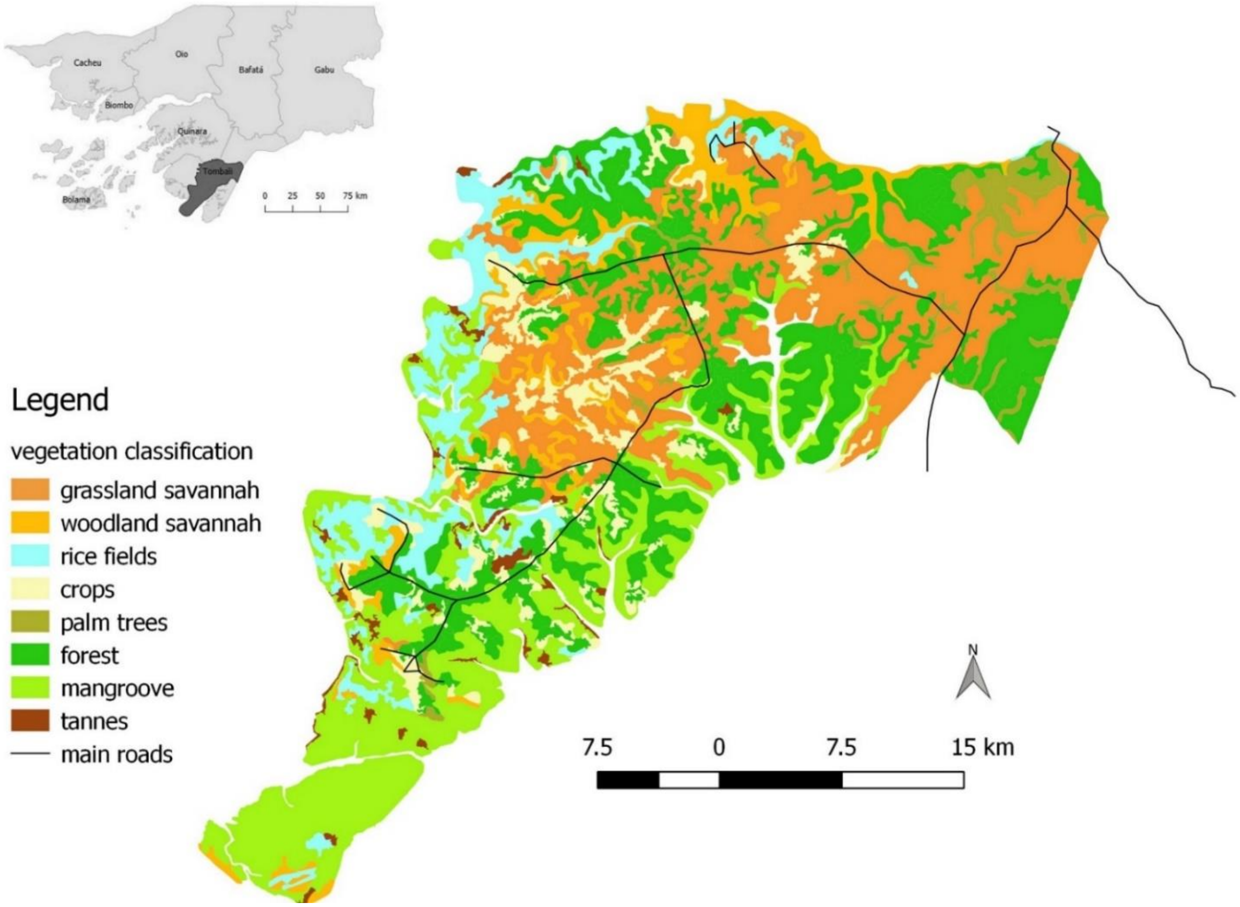


Figure A.1.1 Map of vegetation types in Cantanhez NP.

The north and northwest of the park is predominantly covered by grassland and woodland savanna, while the south and the coastal area of the north of the park comprises more dense forest and mangrove. This map was produced with data provided by (INEP 2013).

APPENDIX 1. Supporting material for CHAPTER 1

Appendix 2 – Supporting material for CHAPTER 2

Script A.2.1 Script semi-structured interviews in Gola RNP.

Questionnaire Sierra Leone 2018

1. Do you use wild plants that have been collected from Gola National Park?
2. Can you tell me the local name of all the wild plants you use?
3. What are they used for? (e.g. medicine, food, religious purposes, etc)
4. Who collects these wild plants in the pristine forest?
5. When do you use these plants? (e.g. celebration days, rainy or dry season, once a month)
6. Are you allowed to collect non-timber forest products (NTFPs) in Gola National Park without asking for permission?
7. Can you find these NTFPs in the Community Forest?
    - 7.1. Yes: Why do you prefer the NTFP from Gola National Park?
    - 7.2. No: Have you ever tried to grow these plant species in the community forest? What happened?

Information of interviewee

1. Gender:
2. Age / Year of birth:
3. Village:
4. Occupation:
5. Education level
6. Religion:

Script A.2.1 Script semi-structured interviews in Cantanhez NP.

Questionário Guiné-Bissau 2019

1. Usa plantas que são/foram recolhidas no parque?
2. Pode dizer o nome das plantas de mato que usa?
3. Para que são usadas? (ex. medicina, alimentação, eventos religiosos, etc)
4. Quem vai à floresta recolher estas plantas de mato?
5. Quando usa as plantas de mato? (ex. dias de celebração, época das chuvas ou seca, etc)
6. Pode encontrar estas plantas de mato em todo o lado?
7. Onde as colhe?
8. Há algum mato/floresta onde é proibido colher plantas de mato?
9. Já tentou plantar estas plantas de mato no seu quintal/lugar/horta? O que aconteceu?

Informação do entrevistado

7. Género:
8. Idade social:
9. Tabanca:
10. Ocupação:
11. Nível de escolaridade:
12. Religião:

Document A.2.1 Information to participants

(first part in English, provided to interviewees in Gola RNP, Sierra Leone; second part in Portuguese, provided to respondents in Cantanhez NP, Guinea-Bissau).

INFORMATION TO PARTICIPANTS

We, hereby, invite you to participate in the PhD project "The use of plants by humans and non-human primates in altered landscapes: diet flexibility or local adaptation?" (FCT fellowship grant SFRH/BD/118444/2016) conducted under the FCT-funded project **PRIMATOMICS** (PTDC/IVC-ANT/3058/2014).

Aims of the project:

The main objective of my PhD project is to evaluate the use of botanic resources found in the National Park, by humans and non-human primates. More specifically, this research aims to (a) use a multi-disciplinary approach to examine multispecies interactions and their impact on socio-ecological systems, and (b) assess the dietary flexibility of non-human primate in fragmented and degraded habitats. Ultimately, this research will contribute to the conservation of the forest, which poses as a vital element to the sustainability of the socio-ecological system involving humans, and endangered chimpanzees and colobus of West Africa.

Project outcomes:

Throughout the development of my PhD project, this research will be to the participants, local partners, at national and international conferences, training courses, a number of academic peer-reviewed publications, some media platforms, and other public activities for the dissemination of the project's results.

Participants in the research project:

Interviewees will be adults specifically selected to suit the objectives of the research. Participants will be informed about project, given the right to remain anonymous and provide their informed consent prior to being interviewed. All researchers (students and academics, park rangers and collaborators, park managers, and other policy makers) involved in the project, as well as interviewees, will be responsible for guaranteeing the confidentiality of both participants and the data provided.

Use and storage of the data provided:

During the oral interviews which will have a duration of approximately 30 to 50 minutes, the researcher will record data that will later be transcribed and coded. Confidentiality and anonymity will be ensured, and access to the information will be restricted and monitored by the PhD student who is conducting the research (Isa Aleixo Pais) and the Principal Investigator of PRIMATOMICS (Dr. Tânia Minhós). The project complies with national legislation concerning personal data protection, and approval by the Ethics Committee of the Research Institute where the project is being developed (CRIA-FCSH Universidade Nova de

Lisboa, Portugal). Personal data will not be used outside the scope of the project and will not be shared with third parties. All personal data will be coded and safely stored in CRIA's repository.

Participants benefits and risks:

The project will provide insight into the importance of wild plants extant in the protected areas, to the livelihood of human communities and the sustainability of endangered non-human primate populations of West Africa. The participants' contribution will help collecting baseline data critical for the improvement of current strategies and the development of news ones that will support the conservation of tropical forests and its endangered species.

Participation in this study is voluntary and there should be no significant risks associated with the interviewee's contribution. If, at any point during the interview, the participant feels in a position of vulnerability, the interview can be interrupted without providing a justification, questions may be asked and withdrawal from the research can take place without any consequences.

Project Funding:

The Portuguese Foundation for Science and Technology (FCT) attributed a PhD fellowship to the student Isa Pais (ref.: SFRH/BD/118444/2016) to develop her project in the Organisms and Environment Division Cardiff University (Wales, U.K.) and Centre for Research in Anthropology (CRIA – NOVA FCSH, Lisboa, Portugal). Part of the field work is financed by the FCT-funded project **PRIMATOMICS** (PTDC/IVC-ANT/3058/2014) coordinated by the Principal Investigator Tânia Minhós.

For more information please contact:

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https://cria.org.pt/en/people/isa-aleixo-pais

INFORMAÇÃO PARA O PARTICIPANTE

Vimos por este modo convidá-lo a participar no projecto de doutoramento “A co-utilização de plantas por parte dos humanos e primatas não-humanos em ambientes alterados: flexibilidade de dieta ou adaptação local” desenvolvido no enquadramento projecto de investigação científica **PRIMATOMICS (**PTDC/IVC-ANT/3058/2014**).**

Objectivos do estudo:

O objectivo principal do meu doutoramento é avaliar o uso de recursos botânicos disponíveis dentro do Parque Nacional, por parte de humanos e primatas não-humanos. Mais especificamente, temos como objectivos (a) usar métodos multi-disciplinares para examinar as interacções entre diversas espécies e o seu impacto num sistema socio-ecológico, e (b) investigar a flexibilidade de dieta dos primatas não-humanos em habitats degradados e fragmentados. Esperamos com este estudo, contribuir para a conservação da floresta que se apresenta como um elemento vital para a sustentabilidade do sistema socio-ecológico entre humanos e espécies ameaçadas de chimpanzés e cólobus na África Ocidental.

Resultados do projecto:

Durante o desenvolvimento do meu projecto, apresentarei os resultados da investigação para as comunidades participantes, parceiros locais, conferências nacionais e internacionais, cursos teorico-prácticos, publicações científicas, plataformas de redes sociais, e outras actividades públicas de dessiminação de resultados.

Participantes no projecto de investigação:

Os participantes serão adultos seleccionados especificamente para responder às questões apresentadas nesta investigação. Os entrevistados serão informados sobre o projecto, permanecerão anónimos e deverão disponibilizar o seu consentimento informado antes de iniciarem a entrevista. Todos os investigadores (estudantes e académicos, guardas, colaboradores e gestores do Parque Nacional, e outros decisores/legisladores) envolvidos no projecto, assim como os participantes, deverão ser responsáveis por garantir a confidencialidade das duas partes e dos dados fornecidos.

Uso e armazenamento dos dados disponibilizados:

Durante a entrevista oral, que terá uma duração máxima de 50 minutos, o investigador registará o meu testemunho que será posteriormente transcrito e codificado. Serão garantidos o anonimato e a confidencialidade, sendo o acesso à informação restrito e monitorizado pela estudante de doutoramento que conduz a referida investigação (Isa Pais) e a investigadora principal do projecto PRIMATOMICS (Dr. Tânia Minhós). O projecto segue as normas de legislação de protecção pessoal de dados, e teve a aprovação do Comité de Ética do Intituto onde decorre a investigação (CRIA – NOVA FCSH). Os dados pessoais não serão usados fora do âmbito do projecto e também não serão partilhados com terceiros. Toda a informação pessoal será armazenada de forma segura e codificada no repositório do CRIA – NOVA FCSH.

Benefícios e riscos para o participante:

O projecto tem como objectivo identificar a importância das plantas silvestres presentes nas áreas protegidas, para a subsistência das comunidades humanas locais e a sustentabilidade de espécies de primatas ameaçados em duas regiões da África Ocidental. O testemunho dos participantes contribuirá para a recolha de conhecimento de base essencial para o melhoramento de actuais e implementação de novas estratégias de conservação de florestas tropicais e primatas não-humanos.

A participação neste estudo será de forma voluntária e não deverão haver riscos associados à contribuição do informador. Se a qualquer momento durante a execução do projecto, o participante se sentir numa posição vulnerável, a entrevista poderá ser interrompida sem qualquer justificação, questões poderão ser colocadas e respondidas por parte da equipa de investigação, e o participante poderá desistir de participar no projecto sem quaiquer consequências para o próprio.

Financiamento do projecto:

A Fundação para a Ciência e a Tecnologia (FCT) atribuiu a bolsa de doutoramento à estudante Isa Pais (ref.: SFRH/BD/118444/2016) com o fim de desenvolver o seu projecto no departamento Organisms and Environment na Universidade de Cardiff (País de Gales, Reino Unido) e no Centro em Rede de Investigação em Antropologia (CRIA – NOVA FCSH, Lisboa, Portugal). Parte do financiamento para trabalho de campo foi obtido a partir do projecto de investigação científica **PRIMATOMICS** financiado pela FCT (PTDC/IVC-ANT/3058/2014) e coordenado pela investigadora principal Dr. Tânia Minhós.

Para mais informações contacte:

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Document A.2.2 Informed consent and debriefing.

The first document was obtained from each respondent that agreed to participate in this research. First version in English used for interviews in Gola RNP, Sierra Leone, and second version in Portuguese used in interviews in Cantanhez NP, Guines-Bissau. At the end of each interview, a debriefing was conducted following the script.

INFORMED CONSENT

The present study is part of a PhD student being developed at the Centre for Research in Anthropology at NOVA FCSH University of Lisbon and the Organisms and Environment Division, Cardiff University. The project “The use of plants by humans and non-human primates in altered landscapes: diet flexibility or local adaptation?” (FCT fellowship grant SFRH/BD/118444/2016) is conducted under the FCT-funded project **PRIMATOMICS** (PTDC/IVC-ANT/3058/2014). The purpose of this research is to produce knowledge on the co-use of botanic resources by humans and non-human primates in two National Parks of West Africa.

This research is being operated by the PhD student Isa Aleixo Pais, supervised by Prof. Michael Bruford (Cardiff University), and Dr Tânia Minhós and Prof. Amélia Frazão Moreira from CRIA - NOVA FCSH.

This interview should last between 30 and 50 minutes.

(1) I confirm that the purpose of the project was explained to me and I had the opportunity to ask questions about the project.

(2) I fully understand that my participation involves answering questions orally, and that my testimony will be later transcribed and coded, with the possibility to be edited upon request.

(3) I am aware that my contribution will remain anonymous and confidential during and after the execution of the project, unless I provide a written consent to disclose the data obtained during my interview.

(3) I recognise that I voluntarily participate in the project and that it is my choice to interrupt or withdraw from the interview at any point in time, with no repercussions to me.

(5) I give permission for data obtained during the interview to be used anonymously in the project’s outcomes.

I agree to participate in this study, coordinated by the PhD student Isa Gameiro Aleixo Pais.

Name and signature of the participant

Name:………………………………………………………………… Signature: ………..……………………………

Date: …. /…./……

Name e signature of the research team’s element

Name:………………………………………………….…………… Signature: …………….………………………….

Date: …. /…./……

DEBRIEFING

Thank you so much for having participated in this study. According to the information provided at the beginning of your participation, this research intends to evaluate the use of botanic resources found in the National Park, by humans and non-human primates. More specifically, this research aims to (a) use a multi-disciplinary approach to examine multispecies interactions and their impact on socio-ecological systems, and (b) assess the dietary flexibility of non-human primate in fragmented and degraded habitats. Ultimately, this research will contribute to the conservation of the forest, which poses as a vital element to the sustainability of the socio-ecological system involving humans, and endangered chimpanzees and colobus of West Africa.

We hereby reinforce your participation in this study will remain anonymous and confidential.

Furthermore, we provide once again the contacts you can use if you wish to pose any question, share any comments, or present your intention of accessing information about the results and findings of the study.

Isa Gameiro Aleixo Pais (PhD student)

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1649-026 Lisbon, Portugal

Thank you once again for your participation in this study.

CONSENTIMENTO INFORMADO

O presente projecto de doutoramento é desenvolvido no Centro em Rede de Investigação em Antropologia (CRIA -NOVA FCSH), e no departamento Organisms and Environment da Universidade de Cardiff. A investigação sobre "A co-utilização de plantas por parte dos humanos e primatas não-humanos em ambientes alterados: flexibilidade de dieta ou adaptação local" (financiamento FCT: SFRH/BD/118444/2016) enquadra-se no projecto de investigação científica **PRIMATOMICS** financiado pela FCT (PTDC/IVC-ANT/3058/2014). Com esta investigação pretendemos obter conhecimento sobre o co-uso de recursos botânicos disponíveis em dois Parques Nacionais na África Ocidental, por parte dos humanos e dos primatas não-humanos (chimanpzés e cólobos vermelhos).

Este estudo é dirigido pela estudante de doutoramento Isa Gameiro Aleixo Pais, e tem a supervisão do Prof. Michael Bruford (Cardiff University), e das investigadoras do CRIA – NOVA FCSH Dr Tânia Minhós e Prof. Amélia Frazão Moreira.

Esta entrevista terá a duração de cerca de 30 a 50 minutos.

(1) Eu confirmo que a equipa de investigação explicou detalhadamente os objectivos do estudo e proporcionou a possibilidade de questionar sobre o projecto.

(2) Eu compreendo inteiramente que a minha participação neste estudo envolve responder a um inquérito oral, e que o meu testemunho será posteriormente transcrito e codificado, tendo a possibilidade de ser editado se assim o requisitar.

(3) Eu entendo que a minha contribuição permanecerá anónima e confidencial durante e após a execução do projecto, e o meu testemunho apenas será disponibilizado no caso de eu providenciar consentimento por escrito.

(3) Eu reconheço que a minha participação neste estudo é voluntária e que é de minha livre vontade interromper ou desistir da entrevista a qualquer momento sem quaiquer repercursões.

(5) Eu dou permissão para que os dados obtidos durante a minha entrevista sejam utilizados de forma anónima na apresentação dos resultados do projecto.

Eu concordo que participei neste estudo, coordenado pela estudante de doutoramento Isa Gameiro Aleixo Pais.

Nome e assinatura do participante

Nome:………………………………………………………………
Assinatura:……………..…………
……………….

Data: …. /…./……

Nome e assinatura do elemento da equipa de investigação

Nome:…………………………………………………….…………… Assinatura: ………………….…………………….

Data: …. /…./……

RESUMO DA PARTICIPAÇÃO

Desde já agradecemos a sua participação neste estudo. De acordo com a informação disponibilizada no início da sua participação, pretendemos com esta investigação, avaliar o uso de recursos botânicos disponíveis dentro do Parque Nacional, por parte de humanos e primatas não-humanos. Mais especificamente, temos como objectivos (a) usar métodos multi-disciplinares para examinar as interacções entre diversas espécies e o seu impacto num sistema socio-ecológico, e (b) investigar a flexibilidade de dieta dos primatas não-humanos em habitats degradados e fragmentados. Esperamos com este estudo, contribuir para a conservação da floresta que se apresenta como um elemento vital para a sustentabilidade do sistema socio-ecológico entre humanos e espécies ameaçadas de chimpanzés e cólobus na África Ocidental.

Gostaríamos de reiterar que a sua participação neste estudo permanecerá anónima e confidencial.

Disponibilizamos novamente os contactos a usar no caso de haver alguma questão que gostasse de ver respondida, partilhar algum comnetário ou apresentar a sua intenção de aceder à informação resultante da execução desta investigação.

Isa Gameiro Aleixo Pais (PhD student)

Centro em Rede de Investigação em Antropologia (CRIA – NOVA FCSH)

Av. Forças Armadas, Edifício ISCTE, sala 2w2

1649-026 Lisbon, Portugal

Mais uma vez, agradecemos a sua participação nesta investigação.

Table A.2.1 Social demography of the sampled population in Gola Rainforest National Park, Sierra Leone.

Forest block	Total FEC villages	FEC villages surveyed	Pop. 2010	No. of respond.	Social demography (values in %)					
					Gender (male)	Elder	Adult	School	Wild plant trading	Miner
Grand totals	**53**	**15**	**3704**	**52**	**63.5**	**67.3**	**32.7**	**26.9**	**7.7**	**9.6**
Gola central	22	10	2349	36	44.2	48.1	21.2	17.3	1.9	9.6
(Gaura and Malema chifdoms)		Bandajuma	109	6	7.7	9.6	1.9	0.0	0.0	3.8
		Gbahama	47	4	3.8	3.8	3.8	3.8	0.0	0.0
		Genneh	363	2	1.9	3.8	0.0	1.9	0.0	0.0
		Gombu	500	1	1.9	0.0	1.9	0.0	0.0	0.0
		Jagolahun	25	6	9.6	7.7	3.8	1.9	0.0	1.9
		Lalehun	600	2	1.9	3.8	0.0	0.0	0.0	0.0
		Nyawama	305	3	3.8	1.9	3.8	1.9	1.9	0.0
		Patama	NA	1	1.9	1.9	0.0	0.0	0.0	0.0
		Takpoima	150	6	7.7	7.7	3.8	5.8	0.0	3.8
		Taiama	250	5	3.8	7.7	1.9	1.9	0.0	0.0
Gola south	31	5	1355	16	19.2	19.2	11.5	9.6	5.8	0.0
(Makpele chiefdom)		Dombu	550	4	5.8	5.8	1.9	5.8	3.8	0.0
		Jeneva	25	2	1.9	1.9	1.9	0.0	1.9	0.0
		Ngekpa	NA	2	3.8	0.0	3.8	1.9	0.0	0.0
		Nyeyama	NA	4	3.8	7.7	0.0	0.0	0.0	0.0
		Pewaa	780	4	3.8	3.8	3.8	1.9	0.0	0.0

FEC: Forest edge community, village situated within the 4km buffer zone (Bulte et al. 2013); Pop. 2010: Community census carried out around Gola RNP in 2010 (Bulte et al 2013); No. of respond.: number of respondents who answered the questionnaire; Gender: percentage of male respondents; Social age: self-reported age cohort or years (men and women) - elder (65^{+} years), adult (between 15 and 64 years); School: people who attended school (Koranic or official school); Wild plant trading: people who sold a portion of the wild plants harvested in the protected area; Miner: people who extracted minerals (mainly gold and diamonds) in the region.

Table A.2.2 Social demography of the sampled population in Cantanhez National Park, Guinea-Bissau.

				Social demography (values in %)						
Chiefdom	**Villages surveyed**	**Pop. 2004**	**No. of respond.**	**Gender (male)**	**Elder**	**Adult**	**Young**	**Community group**	**School**	**Wild plant trading**
Grand totals	**12**	**2315**	**56**	**64.3**	**41.1**	**39.3**	**19.6**		**69.6**	**23.2**
	10	1955	49	61.2	38.8	38.8	22.4		65.3	22.4
	Cabante	75	5	80.0	40.0	40.0	20.0	nl	60.0	40.0
	Cadique	401	4	50.0	0.0	75.0	25.0	nl	100.0	0.0
	Caiquene	85	4	75.0	25.0	25.0	50.0	nl	50.0	25.0
	Cambeque	37	4	50.0	100.0	0.0	0.0	td	50.0	25.0
	Canamine	228	8	50.0	50.0	0.0	50.0	ba, fu, td	75.0	62.5
	Cassintcha	112	3	66.7	0.0	100.0	0.0	ss, bj	100.0	0.0
	Catombói	35	6	66.7	16.7	83.3	0.0	nl	66.7	16.7
	Jemberém	721	3	33.3	66.7	0.0	33.3	td	0.0	0.0
	Lautchande	100	3	100.0	0.0	66.7	33.3	ba	100.0	0.0
	Madina	161	9	55.6	55.6	33.3	11.1	ba, bf, md, ss, fu	55.6	11.1
	2	360	7	85.7	57.1	42.9	0.0		100.0	28.6
	Áfia	226	4	100.0	75.0	25.0	0.0	fu	100.0	25.0
	Quebo-Sutuba	134	3	66.7	33.3	66.7	0.0	fu, ba	100.0	33.3

Pop. 2004: Population census carried out in the country of Guinea-Bissau in 2009 (INE 2017); No. of respond.: total number of respondents who answered the questionnaire; Gender: percentage of respondents that were men; Social age: self-reported age cohort for men and women - elder (*homem grande* and *mulher grande*), adult (*homem* and *mulher*), young (*rapaz* and *bajuda*); Community group: community groups interviewed in the village – ba (balanta), bf (biafada), bj (bijagó), fu (fula), md (mandinga), nl (nalú), ss (sosso), td (tanda); School: people who attended school (Koranic or official school); Wild plant trading: people who sell a portion of the plants collected in the protected area.

Table A.2.3 Wild plant salience score in Gola RNP.

The total number of items listed by all respondents (N = 35) was 49, of which 44 were identified to the species. Information on the plant family, life form, number of times mentioned across interviews, plant names in local languages and the conservation status of each plant is found in the table below.

Plant species	Plant family	Life form	No. times cited	Salience score	Plant common names	Status
Garcinia kola	Clusiaceae	T	19	0.388	sagbei	VU
Massularia acuminata	Rubiaceae	S / T	15	0.329	bush ataya (cr)	LC
Eremospatha spp.	Arecaceae	C	13	0.288	rattan (cr)	LC
Piper guineense	Piperaceae	C	13	0.236	mbahem	LC
Cassia sieberiana	Fabaceae	T / S	10	0.196	gbangbei	LC
Carapa procera	Meliaceae	T	9	0.134	kowi	LC
Eremospatha macrocarpa	Arecaceae	C	7	0.160	balui	LC
Nauclea diderrichii	Rubiaceae	T	7	0.143	bundui	NA
Xylopia aethiopica	Annonaceae	T / S	7	0.118	hewi	LC
Sarcocephalus latifolius	Rubiaceae	S / T	5	0.077	yumbuyabei	NA
Garcinia afzelii	Clusiaceae	T	5	0.074	de nyani	VU
Pentaclethra macrophylla	Fabaceae	T	4	0.066	faawei	LC
Irvingia gabonensis	Irvingiaceae	T	4	0.065	boiboi	NT
Annickia polycarpa	Annonaceae	T	3	0.057	gbelewule	LC
Laccosperma secundiflorum	Arecaceae	C	3	0.033	kavui	LC
Cola lateritia	Malvaceae	T	3	0.031	mbunei	LC
Beilschmiedia mannii	Lauraceae	S / T	3	0.028	walle	LC
Parinari excelsa	Chryssobalanaceae	T	2	0.034	dawei	LC
Macaranga barteri	Euphorbiaceae	S / T	2	0.022	ndewei gboi	LC
Dioscorea cayennensis	Dioscoreaceae	V	2	0.020	mbo	NA

Bambusa vulgaris	Poaceae	G	2	0.014	saemi	NA
Protomegabaria stapfiana	Phyllanthaceae	T	1	0.029	gbogboi	LC
Scleria boivinii	Cyperaceae	C	1	0.026	njaewae	NA
Bussea occidentalis	Fabaceae	T	1	0.026	helei	LC
Pseudospondias microcarpa	Anacardiaceae	T	1	0.023	dorwei	VU
Anisophyllea laurina	Anisophylleaceae	T	1	0.021	kandii	LC
Sida acuta	Malvaceae	S	1	0.016	helui	NA
Albizia zygia	Fabaceae	T	1	0.014	kpakpei	LC
Funtumia africana	Apocynaceae	T	1	0.014	boboe	LC
Ipomoea pileata	Convolvulaceae	H	1	0.014	ndondokoei	NA
Tetracera alnifolia	Dilleniaceae	T	1	0.012	katatie	NA
Dialium dinklagei	Fabaceae	T	1	0.011	ngolomambu	LC
Alstonia boonei	Apocynacecae	T	1	0.010	kalo-wuli	LC
Mareya micrantha	Euphorbiaceae	S / T	1	0.010	nhua.nhua	LC
Tetracera potatoria	Dilleniaceae	T	1	0.010	ndopanei	NA
Dialium guineense	Fabaceae	S / T	1	0.009	mabui	LC
Mammea africana	Calophyllaceae	T	1	0.009	kai.kombei	LC
Anthocleista nobilis	Gentianaceae	T	1	0.007	pongoi	LC
Morinda lucida	Rubiaceae	T	1	0.007	njasui	LC
Raphia spp.	Arecaceae	T	1	0.007	raffia (cr)	LC / DD
Uvaria chamae	Annonaceae	S / T	1	0.007	ndogbo-jele	LC
Sterculia tragacantha	Malvaceae	T	1	0.005	kobei	LC
Morinda morindoides	Rubiaceae	C	1	0.004	kojo logbo	LC
Blighia unijugata	Sapindaceae	T	1	0.002	kenjei	LC

Life form: T = tree, S = shrub, C = climber, V = vine, G = grass, H = herb. No. times cited: number of times an item was mentioned across all interviews. Salience score calculated using the Smith's Index formula in AnthroTools R package (Jamieson-Lane and Purzycki 2016). Plant common names cited in mende or criole (cr). Status - IUCN Red List Conservation status: NA (not available), DD (data deficient), LC (least concern), VU (vulnerable), EN (endangered) (IUCN 2022).

Table A.2.4 Wild plant salience score in Cantanhez NP.

The total number of items listed by all respondents (N = 56) was 120, of which 82 were identified to the species. Information on the plant family, life form, number of times mentioned across interviews, plant names in local languages and the conservation status of each plant is found in the table below.

Plant species	Plant family	Life form	No. times cited	Salience score	No. uses reported	Plant common names	Status
Dialium guineense	Fabaceae	S / T	33	0.361	4	veludo (cr)	NA*
Anisophyllea laurina	Anisophylleaceae	T	28	0.348	4	miséria (cr)	LC
Parinari excelsa	Chrysobalanaceae	T	26	0.324	5	manpatace (cr)	LC
Elaeis guineensis	Arecaceae	T	24	0.254	4	palmera (cr)	LC
Cassia sieberiana	Fabaceae	T / S	23	0.255	4	canafistra (cr)	LC
Uvaria chamae	Annonaceae	S / T	15	0.194	3	banana-de-santcho (cr)	LC
Pterocarpus erinaceus	Fabaceae	T	15	0.158	3	pau-sangue (cr)	EN
Spondias mombin	Anacardiaceae	T / S	11	0.101	2	mandiple (cr)	LC
Sarcocephalus latifolius	Rubiaceae	S / T	11	0.100	1	madronho (cr)	NA
Parkia biglobosa	Fabaceae	T	11	0.077	3	farroba (cr)	LC
Khaya senegalensis	Meliaceae	T	10	0.113	3	bissilon (cr)	VU
Avicennia germinans	Acanthaceae	S / T	10	0.093	3	tarrafe (cr)	LC
Landolphia sp.	Apocynaceae	S / T	8	0.083	2	fole (cr)	VU
Ceiba pentandra	Malvaceae	T	8	0.080	3	poilão (cr)	LC
Combretum micranthum	Combretaceae	S / T	8	0.074	2	buco (cr)	LC
Neocarya macrophylla	Chrysobalanaceae	T / S	8	0.058	2	tambacumba (cr)	NA
Xylopia aethiopica	Annonaceae	T / S	6	0.080	0	malagueta-preta (cr)	LC
Alstonia boonei	Apocynaceae	T	6	0.072	2	tagara (cr)	LC
Adansonia digitata	Malvaceae	T	6	0.052	2	cabaceira (cr)	NA
Landolphia heudelotii	Apocynaceae	S / T	6	0.048	1	fole-di-lala (cr)	NA
Hunteria umbelata	Apocynaceae	T	5	0.071	0	pó-di-pinti (cr)	LC

Zanthoxylum zanthoxyloides	Rutaceae	S	5	0.066	0	cancansa (ss)	LC
Strophanthus hispidus	Apocynaceae	S / C	5	0.058	1	malila (cr)	LC
Anthocleista procera	Gentianaceae	T	5	0.053	2	caboupa-matcho (cr)	LC
Afzelia africana	Fabaceae	T	5	0.048	3	pó-de-conta (cr)	VU
Saba senegalensis	Apocynaceae	C	5	0.044	0	fole-elefante (cr)	NA
Borassus aethiopum	Arecaceae	T	4	0.042	1	cibe (cr)	LC
Albizia adianthifolia	Fabaceae	T	4	0.041	2	farroba-de-mato (cr)	LC
Schrebera arborea	Oleaceae	T	4	0.037	1	pau-goiaba (cr)	LC
Bauhinia reticulata	Fabaceae	S / T	4	0.030	1	ápéss (cr)	NA
Calycobolus heudelotii	Convolvulaceae	C	4	0.025	1	manar-fana (nl)	NA
Trema orientalis	Cannabaceae	S	3	0.046	0	quere (fu)	LC
Strombosia pustulata	Olacaceae	T	3	0.045	1	osso-de-dari (cr)	LC
Ficus sur	Moraceae	T	3	0.042	2	tonkin-iá (nl)	LC
Vitex doniana	Lamiaceae	T / S	3	0.031	1	azeitona (cr)	LC
Antiaris toxicaria	Moraceae	T	3	0.029	1	pau-bicho (cr)	LC
Strophanthus sarmentosus	Apocynaceae	S / C	3	0.023	0	quindé (cr)	NA
Caesalpinia benthamiana	Fabaceae	S / T	3	0.010	1	tepedarola (cr)	NA
Albizia zygia	Fabaceae	T	3	0.010	3	pó-de-raio (cr)	LC
Newbouldia laevis	Bignoniaceae	S	3	0.010	1	manducu-de-feticero (cr)	NA
Securidaca longipedunculata	Polygalaceae	S / T	2	0.025	1	djutu (bf)	NA
Calamus deerratus	Arecaceae	S / T	2	0.021	0	mantampa-de-sera (cr)	LC
Zanthoxylum leprieurii	Rutaceae	T / S	2	0.021	0	barquelem (fu)	NA
Psychotria peduncularis	Rubiaceae	S	2	0.019	1	átamul (td)	LC
Sorindeia juglandifolia	Anacardiaceae	T / S	2	0.017	0	ambilire (td)	LC
Holarrhena floribunda	Apocynaceae	S / T	2	0.015	0	bribait (ba)	LC
Senna podocarpa	Fabaceae	S	2	0.013	1	atchapide (cr)	NA
Morinda chrysorhiza	Rubiaceae	T / S	2	0.013	1	dacuré (fu)	NA
Laguncularia racemosa	Combretaceae	S	2	0.011	0	n'concom (ss)	LC
Raphia palma-pinus	Arecaceae	T	2	0.011	0	ápél (cb)	NT
Bauhinia thonningii	Fabaceae	S / T	2	0.011	1	pano-di-kankora (cr)	NA

Ficus sp.	Moraceae	T / S	2	0.009	1	ficus (cr)	LC / NT
Lophira lanceolata	Ochnaceae	T	2	0.004	1	mené (cr)	LC
Hymenocardia acida	Phyllanthaceae	S / T	2	0.004	1	matikzé (cr)	LC
Daniellia oliveri	Fabaceae	T	1	0.018	0	boto (nl)	LC
Oxytenanthera abyssinica	Poaceae	G	1	0.018	0	cana-bambu (cr)	NA
Sterculia tragacantha	Malvaceae	T	1	0.018	0	mandundufe (nl)	LC
Detarium microcarpum	Fabaceae	T	1	0.016	0	boto (fu)	LC
Bridelia micrantha	Phyllanthaceae	T / S	1	0.015	1	tolingué (ss)	NA
Voacanga africana	Apocynaceae	S	1	0.015	0	pó-de-braço (cr)	NA
Blighia unijugata	Sapindaceae	T	1	0.015	0	firifora (md)	LC
Antidesma membranaceum	Phyllanthaceae	S / T	1	0.013	0	quere (fu)	LC
Craterispermum laurinum	Rubiaceae	S	1	0.012	1	landam-édi (fu)	NA
Ficus exasperata	Moraceae	T	1	0.012	1	língua-di-baca (cr)	LC
Lannea acida	Anacardiaceae	T	1	0.011	1	tchingole (fu)	LC
Annona senegalensis	Annonaceae	S	1	0.009	0	anocotô (td)	LC
Anthostema senegalense	Euphorbiaceae	T	1	0.008	0	mante (nl)	LC
Klainedoxa gabonensis	Irvingiaceae	T	1	0.008	0	n'bámbete (nl)	LC
Schoenoplectus litoralis	Cyperaceae	H	1	0.008	0	inthué (ba)	LC
Carapa procera	Meliaceae	T	1	0.007	0	siti-malgos (cr)	LC
Aframomum sp.	Zingiberaceae	H	1	0.006	0	belencufa (cr)	LC
Dichrostachys cinerea	Fabaceae	T / S	1	0.006	1	fedida-branco (cr)	LC
Phyllanthus muellerianus	Phyllanthaceae	T / S	1	0.006	1	mámámómóti (fu)	NA
Erythrina senegalensis	Fabaceae	T / S	1	0.004	1	pó-de-osso (cr)	LC
Terminalia macroptera	Combretaceae	T / S	1	0.004	0	macite (cr)	LC
Pterocarpus santalinoides	Fabaceae	T / S	1	0.003	0	d'jego (cr)	LC
Detarium senegalense	Fabaceae	T	1	0.003	0	mambode (cr)	LC
Imperata cylindrica	Poaceae	H	1	0.003	1	sódjô (fu)	LC
Allophylus africanus	Sapindaceae	S	1	0.001	1	futété (ss)	LC
Cyperaceae (Fam.)	Cyperaceae	H	1	0.002	0	mampufa (cr)	LC

Hexalobus monopetalus	Annonaceae	T / S	1	0.002	0	boile (fu)	LC
Pentaclethra macrophylla	Fabaceae	T	1	0.002	0	n'tantass (ba)	LC

Life form: T = tree, S = shrub, C = climber, V = vine, G = grass, H = herb. No. times cited: number of times an item was mentioned across all interviews. Salience score calculated using the Smith's Index formula in AnthroTools R package (Jamieson-Lane and Purzycki 2016). Plant common names cited in local languages: ba (balanta), bf (biafada), cs (cobiana), cr (crioulo), fu (fula), md (mandinga), nl (nalú), ss (sosso), td (tanda). Status - IUCN Red List Conservation status: NA (not available), DD (data deficient), LC (least concern), VU (vulnerable), EN (endangered) (IUCN 2022). NA* no assessment at IUCN, but POWO (2022)has a not threatened record.

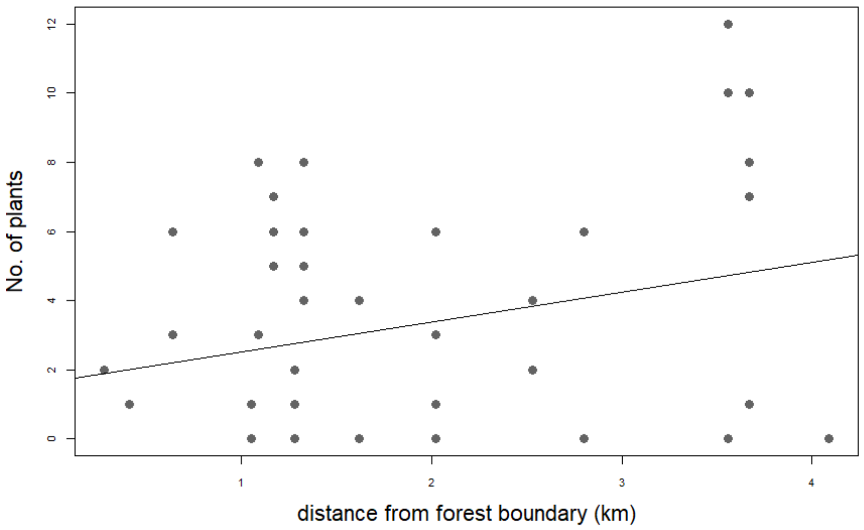


Figure A.2.1 Linear regression analysis for the complete dataset in Gola RNP.

Test for correlation between distance from the FEC where the respondent lives to the protected forest boundary, and the number of items freelisted by the interviewee; This analysis was performed with the entire dataset (N = 52, respondents who did not use wild plants were assigned the value “zero” in the number of plants listed) but the apparent positive correlation observed is not significant (S = 18748, N = 52, $p > 0.05$). Interviews were conducted in the dry and rainy seasons.

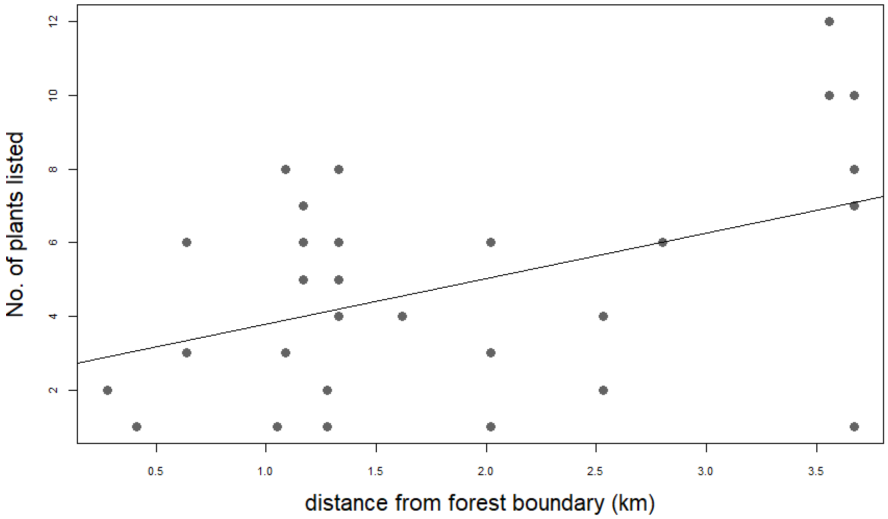


Figure A.2.2 Linear regression analysis for respondents using wild plants.

Test for correlation between distance from the FEC where the respondent lives to the protected forest boundary, and the number of items freelisted by the interviewee. In this analysis, only respondents who claimed to use wild plants collected in the protected forest were used (N = 35). Interviews were conducted in the dry and rainy seasons. The positive correlation observed is significant (r = 0.456, N = 35, d.f. = 33, p = 0.006).

Appendix 3 – Supporting material for CHAPTER 3

Document A.3.1 DNA extraction protocol for primate faecal samples.

QIAamp Fast DNA Stool Mini Handbook 03/2014

Alterations done by Isa Aleixo Pais & Filipa Borges

Protocol: Isolation of DNA from Stool for Human DNA Analysis

Lysis conditions in this protocol are optimized to increase the ratio of human DNA to nonhuman DNA. Nonhuman DNA is not excluded by this protocol.

Important points before starting

All centrifugation steps should be carried out at room temperature (15–25°C) at 20,000 x g (approximately 14,000 rpm). Increase the centrifugation time proportionately if your centrifuge cannot provide 20,000 x g (e.g., instead of centrifuging for 5 min at 20,000 x g, centrifuge for 10 min at 10,000 x g).

Things to do before starting

- Prepare a thermomixer with 2 ml inlays or a water bath at 70°C for use in step 7
- Redissolve any precipitates in Buffer AL and InhibitEX Buffer by incubating at 37–70°C
- Add ethanol to Buffer AW1 and Buffer AW2 concentrates
- Mix all buffers before use

Procedure

1. Cut the surface layer of the faecal sample and weigh 180–220 mg stool in a 2 ml microcentrifuge tube (not provided).

Note: It is preferrable to prepare a duplicate tube at the same time, so you can start the extraction from the next point onwards, in case your ran out of DNA. For this reason, the tube should be labelled with the original code!!

This protocol is optimized for use with 180–220 mg stool but can also be used with smaller amounts. There is no need to reduce the amounts of buffers when using smaller amounts of stool. For samples >220 mg, see “Protocol: Isolation of DNA from Larger Volumes of Stool”, page 26. If the sample is liquid, pipet 200 µl into the microcentrifuge tube. Cut the end of the pipet tip to make pipetting easier. If the sample is frozen, use a scalpel or spatula to scrape bits of stool into a 2 ml microcentrifuge tube on ice.

Note: When using frozen stool samples, take care that the samples do not thaw until InhibitEX Buffer is added in step 2 to lyse the sample; otherwise the DNA in the sample may degrade. After addition of InhibitEX Buffer, all following steps can be performed at room temperature (15–25°C).

2. Add 1.4 ml InhibitEX Buffer to each stool sample. Mash the sample with a pestle and incubate overnight at 56ºC, 900rpm.

Note: It is important to vortex the samples thoroughly. This helps ensure maximum DNA concentration in the final eluate.

3. Centrifuge sample at full speed for 3 min to pellet stool particles.

IMPORTANT: Do not transfer any solid material. If particles are still visible in the supernatant, centrifuge the sample again.

4. Pipet 25 µl proteinase K into a new 2 ml microcentrifuge tube (not provided).

NOTE: You can use numbers 1 to 24 (as many as you can fit in the centrifuge) to label the tubes. Make sure you have noted the list of samples you are extracting in your lab book.

5. Pipet 600 µl supernatant from step 3 into the 2 ml microcentrifuge tube containing proteinase K.

6. Add 600 µl Buffer AL and vortex for 15 s.

Note: Do not add proteinase K directly to Buffer AL. It is essential that the sample and Buffer AL are thoroughly mixed to form a homogeneous solution.

7. Incubate at 70°C for 30 min. Centrifuge briefly to remove drops from the inside of the tube lid (optional).

8. Add 600 µl of ethanol (96–100%) to the lysate, and mix by vortexing. Centrifuge briefly to remove drops from the inside of the tube lid (optional).

9. Carefully apply 600 µl lysate from step 8 to the QIAamp spin column. Close the cap and centrifuge at full speed for 1 min. Discard filtrate and use the same collection tube.

10. Repeat step 9 until all of the lysate has been loaded on the column. Close each spin column in order to avoid aerosol formation during centrifugation. If the lysate has not completely passed through the column after centrifugation, centrifuge again until the QIAamp spin column is empty.

11. Carefully open the QIAamp spin column and add 500 µl Buffer AW1. Centrifuge at full speed for 1 min. Place the QIAamp spin column in a new 2 ml collection tube, and discard the collection tube containing the filtrate.

12. Carefully open the QIAamp spin column and add 500 µl Buffer AW2. Centrifuge at full speed for 3 min. Discard the collection tube containing the filtrate.
Note: Residual Buffer AW2 in the eluate may cause problems in downstream applications. Some centrifuge rotors may vibrate upon deceleration, causing the flow-through containing Buffer AW2 to come in contact with the QIAamp spin column. Removing the QIAamp spin column and collection tube from the rotor may also cause flow-through to come in contact with the QIAamp spin column.

13. Place the QIAamp spin column in a new 2 ml collection tube (not provided) and discard the old collection tube with the filtrate. Centrifuge at full speed for 3 min. This step helps to eliminate the chance of possible Buffer AW2 carryover.

14. Transfer the QIAamp spin column into a new, labeled 1.5 ml microcentrifuge tube (not provided)

NOTE: Label the final tube which will contain the DNA samples, with the original code.

and pipet 100 µl Buffer ATE directly onto the QIAamp membrane. Incubate for 10 min at room temperature, then centrifuge at full speed for 3 min to elute DNA. If yield is to be quantified by UV absorbance, blank the measuring device using Buffer ATE to avoid false results. For more information about elution and how to determine DNA yield, purity, and length, see the Appendix on page 33. For long-term storage, we recommend keeping the eluate at –20°C.

Table A.3.1 Dietary studies on chimpanzees (*Pan troglodytes*).

For many studies sample size is not available as data was obtained mainly through direct observations.

(sub)Species	Author	Site	Study period	Method	Plant spp. consumed	Sample size
P. t. schweinfurthii	(Nishida and Uehara 1983)	Mahale Mountains, Tanzania	1965-1981	O/F	198	-
P. t. verus	(McGrew et al. 1988)	Mt. Assirik, Senegal	Feb 1976 - Dec 1979	O/F/T	43	-
P. t. verus	(Sugiyama and Koman 1992)	Bossou, Guinea	1976-1992	O	203	-
P. t. troglodytes	(Tutin et al. 1997)	Lopé Reserve, Gabon	10 yrs	O/F	182	
P. t. verus	(Yamakoshi 1998)	Bossou, Guinea	2 years	O	110	-
P. t. schweinfurthii	(Wrangham et al. 1998)	Kibale NP, Uganda	1992-1993	O	32 (Fr)	-
P. t. verus	(Takemoto 2003)	Bossou, Guinea	1995-1998	O	52 (T)	-
P. t. verus	(Pruetz 2006)	Fongoli, SouthE Senegal	3 years	O/F/T	47+	1007
P. t. verus	Hockings et al.	Bossou, Guinea	12 months	O	140 (123WP + 17CR)	-
P. t. schweinfurthii	(Chancellor et al. 2012)	Rwanda	12 months	O/F	23 (Fr)	-

P. troglodytes	(Hockings and McLennan 2012)	27 sites (10 African countries)	1931-2011	O / F / T / R	36 (CR)	various
P. t. schweinfurthii	(McLennan 2013)	Bulindi, Uganda	13 months	T/F/O	82 (71WP + 11CR)	-
P. t. verus	(Bessa et al. 2015)	CNP, Guinea-Bissau	9 months	T/F/O	66 (57WP + 9CR)	377
P. t. schweinfurthii	(Piel et al. 2017)	Issa valley, western Tanzania	4yrs	F	69 +	-
P. t. ellioti	(Dutton and Chapman 2015)	Ngel Nyaki Forest Reserve, Nigeria	12 months	T/F/O	52 (Fr)	-
P. t. schweinfurthii	(McLennan et al. 2020)	Bulindi, Uganda	13 months + 14 months	F	119	1,918

Method: direct observations (O), faecal analysis (F), feeding traces (T), people's reports (R). Plant species consumed: wild plants (WP), crops (CR), fruits (Fr), trees (T)

Appendix 4 – Supporting material for CHAPTER 4

Figure A.4.1 Temminck’s red colobus sample location from 2009.

The red dots refer to the samples from groups of red colobus collected in the same forest fragments as in the current study. Source: Minhós 2012.

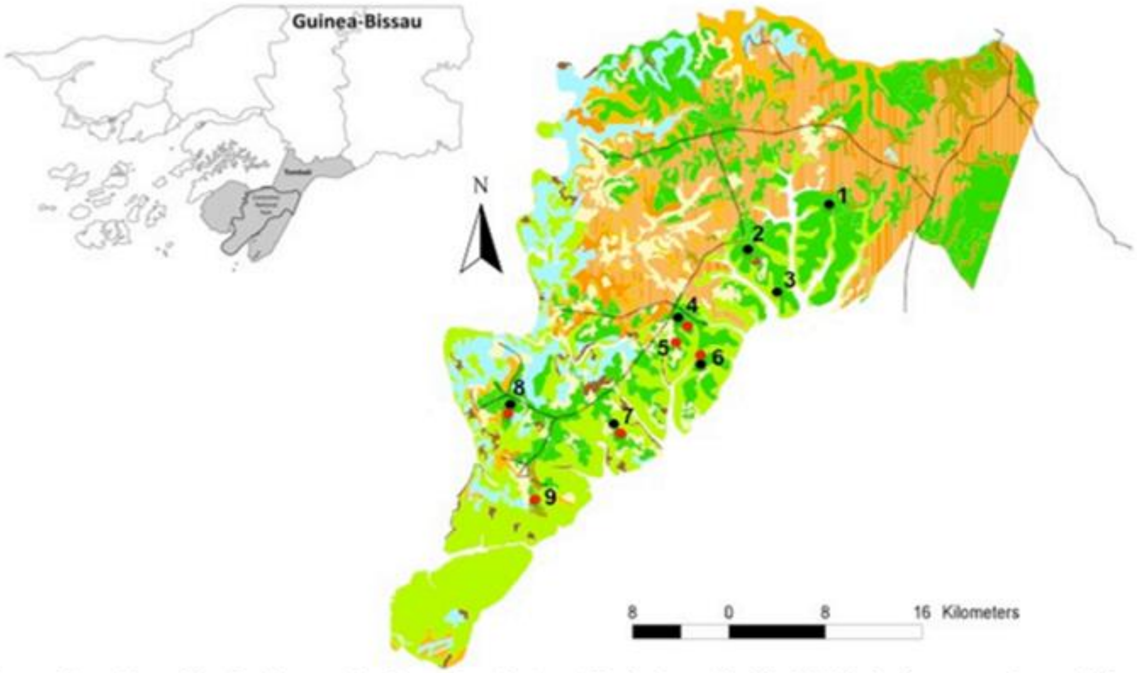


Figure 2.1 – Map of the land cover for Cantanhez National Park (provided by INEP): dark green – forest; light green – mangrove; yellow – savanna; blue – rice fields; beige – crops; brown – tannes. Black circles: black-and-white colobus sampled groups; red circles: red colobus sampled groups. 1 – Cancira; 2 – Amidara; 3 – Deep Amidara; 4 – Focal and Neighbor; 5 - Madina; 6 – Cambeque; 7 - Cangode; 8 – Muna; 9 – Cungha.

Table A.4.1 Dietary studies on red colobus (*Pilicolobus*).

These studies used the traditional methods used in feeding ecology, direct observations, and morphological examination of plant parts in the faecal matter. The number of plants consumed refer to those for which identification was achieved. In general, the number of plants consumed were higher but taxonomic identification was not possible for some items. Top plants are the ones for which primates spend more time feeding on or more frequently encountered in the faeces.

Red colobus	**Year**	**Site**	**Study period in years**	**Plant species consumed**	**Top plants**	**Study**
P. b. badius	1999	Tiwai, Sierra Leone	1	47	*Funtumia Africana* *Amphimas pterocarpioides* *Detarium senegalense*	(Davies et al. 1999)
	2010-2014	Tai, Côte d'Ivoire	< 2	32	*Caloncoba brevipes* *Strephonema pseudocola*	(McGraw et al. 2016)
	2017	Tai, Côte d'Ivoire	0.6	34	*Scytopetalum tieghemii* *Lophira alata*	(Wilkins 2017)
P. b. temminckii	1977 / 1978	Abuko, The Gambia	1	39	-	(Gatinot 1977; Gatinot 1978)
	1991	Abuko, The Gambia	1	89	-	(Starin 1991)
P. kirkii	1991 / 1993	Jozani, Tanzania	1	62-63	-	(Mturi 1991; Mturi 1993)
P. preussi	2001	Korup, Cameroon	1.3	17	-	(Usongo and Amubode 2001)

P. rufomitratus	1994-1999 / 2006 -2007	Kibale, Uganda	1.7	31	*Celtis durandii* *Celtis africana*	(Ryan et al. 2013)
	1981	Tana River, Tanzania	1.3	22	*Ficus sycomorus* *Sorindeia excelsa* *Strombosia scheffleri*	(Marsh 1981)
P. tephrosceles	1970-1972	Kibale, Uganda	1.6	46	*Celtis africana*	(Struhsaker and Oates 1975)
	1972 - 1987	Kibale, Uganda	9.9	85	*Celtis africana*	(Struhsaker 2010)
	1976 - 1983	Kibale, Uganda	3.8	70	*Morus lacteal* *Newtonia buchananii*	(Struhsaker 2010)
	1975	Gombe, Tanzania	0.9	>58	*Celtis durandii* *Parinari excelsa* *Stombosia scheffleri*	(Clutton-Brock 1975)
	2011	Mbuzi, Tanzania	0.2	36	*Parinari excelsa*	(Kibaja 2014)
P. tholloni	1994	Botsima, Zaire	1	84	-	(Maisels et al. 1994)

Table A.4.2 Complete list of plant taxa detected in the diet of Bay colobus (*P. b. badius*).

Characteristics of wild and crop plant taxa detected in *P. b. badius* non-invasive samples using a DNA metabarcoding approach.

Plant family	**Dietary taxon**	**Life form**	**Season**	**F_o(%)**	**Status**	**Part eaten**
Ochnaceae	*Lophira alata*	Tree	D / R	37.20	VU	-
Fabaceae	*Anthonotha* spp.	Tree	D / R	36.71	LC	YL
Combretaceae	*Combretum* spp.	Shrub	D / R	33.82	LC	Fl / ML / S / YL
Chrysobalanaceae	*Maranthes aubrevillei*	Tree	D / R	33.82	NA	-
Rubiaceae	*Nauclea* spp.	Tree	R	21.26	NT / LC	Fl / YL
Clusiaceae	*Pentadesma butyracea*	Tree	D / R	17.39	LC	-
Moraceae	*Ficus* spp.	Tree	D / R	16.91	LC	Fr / ML / YL
Fabaceae	*Didelotia idae*	Tree	D / R	15.94	NT	-
Euphorbiaceae	*Manniophyton fulvum*	Shrub / Climber	D / R	15.46	NA	ML / YL
Rubiaceae	*Keetia* spp.	Tree / Shrub	D / R	14.98	LC	-
Meliaceae	*Carapa procera*	Tree	D / R	14.49	LC	-
Sapotaceae	*Manilkara obovata*	Tree	D / R	13.53	LC	-
Dilleniaceae	*Tetracera* spp.	Tree	D / R	12.56	NA	-
Meliaceae	*Trichilia monadelpha*	Tree / Shrub	D / R	12.08	LC	Fl / ML / YL
Myrtaceae	*Eugenia* spp.	Tree / Shrub	D / R	11.59	LC	-
Combretaceae	*Strephonema pseudocola*	Shrub / Tree	D / R	11.11	LC	-
Loganiaceae	*Strychnos* spp.	Shrub / Tree	D / R	10.63	NA	Fl / YL
Combretaceae	*Terminalia* spp.	Tree / Shrub	D / R	8.21	VU	Fl / S / YL

Phyllanthaceae	*Bridelia micrantha*	Tree / Shrub	D / R	7.73	LC	YL
Fabaceae	*Cryptosepalum tetraphyllum*	Tree	D	7.25	VU	-
Fabaceae	*Dalbergia* spp.	Tree / Shrub / Climber	D / R	5.80	LC	-
Fabaceae	*Daniellia ogea*	Tree	R	4.35	NT	Fl / YL
Meliaceae	*Heckeldora leonensis*	Tree	D / R	3.86	LC	-
Urticaceae	*Myrianthus arboreus*	Tree	D / R	3.86	LC	IS / ML / YL
Polygonaceae	*Afrobrunnichia erecta*	Vine	D / R	3.38	NA	-
Euphorbiaceae	*Macaranga barteri*	Shrub / Tree	D / R	3.38	LC	YL
Chrysobalanaceae	*Parinari* spp.	Tree	D / R	3.38	LC	Fl / Fr / ML / YL
Melastomataceae	*Memecylon* spp.	Shrub / Tree	R	2.90	LC	-
Malvaceae	*Rhodognaphalon* spp.	Tree	R	2.90	VU	-
Sterculiaceae	*Cola* spp.	Tree	D	2.42	NT / LC / VU	ML / YL
Malpighiaceae	*Acridocarpus plagiopterus*	Shrub	R	1.93	LC	-
Fabaceae	*Aganope leucobotrya*	Shrub / Tree	R	1.93	LC	-
Fabaceae	*Berlinia confusa*	Tree	R	1.93	LC	ML
Rubiaceae	*Coffea* spp. ©	Shrub / Tree	D / R	1.93	LC	-
Fabaceae	*Gilbertiodendron preussii*	Tree	D	1.93	LC	-
Sapotaceae	*Omphalocarpum pachysteloides*	Tree / Shrub	R	1.93	LC	-
Fabaceae	*Paramacrolobium coeruleum*	Tree	D / R	1.93	LC	-
Fabaceae	*Brachystegia leonensis*	Tree	R	1.45	VU	-
Chrysobalanaceae	*Dactyladenia smeathmannii*	Shrub	R	1.45	EN	-
Rhizophoraceae	*Anisophyllea meniaudi*	Tree	R	0.97	NA	ML

Fabaceae	*Enterolobium* spp. ©	Tree	D / R	0.97	LC	-
Celastraceae	*Helictonema velutinum*	Vine	R	0.97	NA	-
Lecythidaceae	*Napoleonaea* spp.	Shrub / Tree	R	0.97	LC	-
Simaroubaceae	*Odyendea klaineana*	Tree	D / R	0.97	NA	Fl / ML / S / YL
Lamiaceae	*Vitex* spp.	Tree / Shrub	D / R	0.97	LC	Fl / YL
Celastraceae	*Campylostemon* spp.	Vine / Shrub	R	0.48	LC	Fl / ML / YL
Sapindaceae	*Chytranthus* spp.	Tree / Shrub	R	0.48	LC	-
Rubiaceae	*Craterispermum caudatum*	Shrub	R	0.48	LC	-
Rubiaceae	*Fleroya* spp.	Tree	R	0.48	NT	-
Apocynaceae	*Funtumia africana*	Tree	D	0.48	LC	Fl / Fr / S / YL
Clusiaceae	*Garcinia afzelli*	Tree / Shrub	R	0.48	VU	YL
Rubiaceae	*Hutchinsonia barbata*	Shrub	R	0.48	NA	-
Fabaceae	*Leptoderris* spp.	Climber	D	0.48	LC	-
Meliaceae	*Lovoa trichilioides*	Tree	R	0.48	LC	Fr / ML / YL
Euphorbiaceae	*Macaranga heterophylla*	Shrub / Tree	R	0.48	LC	-
Sapotaceae	*Malacantha alnifolia*	Shrub / Tree	D	0.48	NA	-
Urticaceae	*Musanga cecropioides*	Tree	R	0.48	LC	-
Rubiaceae	*Mussaenda* spp.	Shrub	R	0.48	NA	-
Fabaceae	*Plagiosiphon emarginatus*	Tree / Shrub	R	0.48	LC	Fl / ML / S / YL
Fabaceae	*Platysepalum hirsutum*	Trees / Shrubs / Vines	R	0.48	NA	-
Menispermaceae	*Rhigiocarya racemifera*	Vine	R	0.48	NA	-
Cucurbitaceae	*Ruthalicia eglandulosa*	Climber	R	0.48	NA	-

Life form: climber (C), shrub (S), tree (T). Season: dry (D) Dec-Apr; rainy (R) May-Nov. F_o(%): Frequency of occurrence, the number of times plant taxa was detected across samples (N = 207). Part eaten: flower (Fl), fruit (Fr, mostly unripe), mature leaves (ML), young leaves (YL), seeds (S); this information was obtained from previous studies (references in Table A.4.1 in Appendix 4). © crops. Status: IUCN Red List Conservation status, least concerned (LC), near threatened (NT), vulnerable (VU), not available (NA).

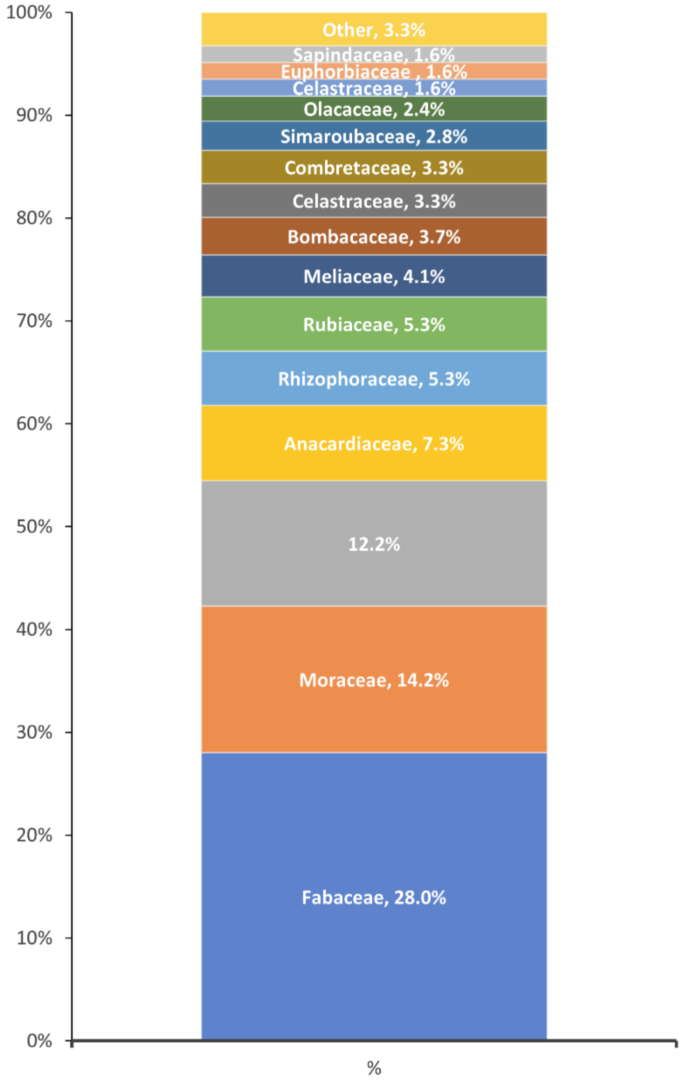


Figure A.4.2 Cantanhez NP red colobus dietary composition in 2009 by plant family.

The frequency of occurrence of plants detected in *P. b. temminckii* diet per plant family was calculated using dietary counts. Three families contributing to more than 50% of the dietary requirements of this primate species in Catanhez NP. For visualisation purposes, six additional plant families were grouped together under the category ‘Other’. These taxa were detected in 1% or less of all 207 samples. The data presented here refers to the 2009 dataset.

Table A.4.3 Complete list of plant taxa detected in the diet of Temminck's red colobus (*P. b. temminckii*) in 2019. Characteristics of wild and crop plant taxa detected in *P. b. temminckii* non-invasive samples using a DNA metabarcoding approach.

Plant family	**Dietary taxon**	**Life form**	**Season**	**F_o(%)**	**Status**	**Plant part eaten**
Moraceae	*Treculia africana*	Tree	D / R	51.49	LC	ML / YL
Fabaceae	*Leptoderris* spp.	Climber	D / R	39.60	LC	-
Chrysobalanaceae	*Parinari excelsa*	Tree	D / R	39.60	LC	Fl / Fr / ML / YL
Moraceae	*Ficus* spp.	Tree	D / R	38.61	LC	Fr / ML / YL
Sapotaceae	*Malacantha alnifolia*	Shrub / Tree	D / R	34.65	NA	-
Combretaceae	*Combretum* spp.	Shrub	D / R	26.73	LC	Fl / ML / S / YL
Rubiaceae	*Ixora* spp.	Shrub	D / R	25.74	LC	-
Fabaceae	*Cassia sieberiana*	Tree / Shrub	D / R	24.75	LC	-
Dilleniaceae	*Tetracera potatoria*	Tree	D / R	22.77	NA	-
Avicenniaceae	*Avicennia germinans*	Tree / Shrub	D / R	18.81	LC	-
Hypericaceae	*Harungana madagascariensis*	Tree / Shrub	D / R	17.82	LC	-
Rubiaceae	*Uncaria africana*	Shrub	R	16.83	NA	-
Bombacaceae	*Ceiba pentandra*	Tree	D	15.84	LC	-
Fabaceae	*Mezoneuron* spp.	Vine	D / R	14.85	NA	-
Celastraceae	*Simicratea welwitschii*	Climber	D	13.86	NA	-
Fabaceae	*Dichrostachys cinerea*	Tree / Shrub	R	12.87	LC	Fl / ML / YL
Moraceae	*Milicia regia*	Tree	D / R	12.87	VU	-

Euphorbiaceae	*Phyllanthus muellerianus*	Shrub / Vine	D / R	12.87	NA	ML / YL
Meliaceae	*Trichilia prieureana*	Tree	D	12.87	LC	Fl / ML / YL
Fabaceae	*Crudia senegalensis*	Shrub	R	8.91	NA	-
Anacardiaceae	*Mangifera indica* ©	Tree	D	8.91	DD	-
Rubiaceae	*Keetia venosa*	Tree / Shrub	D / R	5.94	LC	-
-Meliaceae	*Trichilia monadelpha*	Tree / Shrub	D / R	5.94	LC	Fl / ML / YL
Olacaceae	*Ximenia americana*	Shrub / Tree	D / R	4.95	LC	-
Malpighiaceae	*Acridocarpus plagiopterus*	Shrub	D	3.96	LC	-
Rubiaceae	*Coffea* spp. ©	Shrub / Tree	D / R	3.96	LC	-
Euphorbiaceae	*Macaranga heterophylla*	Shrub / Tree	D	3.96	LC	-
Sapindaceae	*Paullinia pinnata*	Climber	D	3.96	NA	-
Sapotaceae	*Mimusops adongensis*	Tree	R	2.97	LC	Fl / Fr / ML / YL
Simaroubaceae	*Odyendea* spp.	Tree	D	2.97	NA	Fl / ML / S / YL
Meliaceae	*Carapa procera*	Tree	R	1.98	LC	-
Rubiaceae	*Cremaspora triflora*	Shrub	R	1.98	LC	-
Fabaceae	*Dalbergia* spp.	Tree / Shrub / Climber	D	1.98	LC	-
Chrysobalanaceae	*Neocarya macrophylla*	Tree	D	1.98	NA	-
Fabaceae	*Pterocarpus* spp.	Tree	D	1.98	EN	Fl / Fr / ML / YL
Celastraceae	*Reissantia indica*	Shrub	D / R	1.98	NA	Fl / Fr / ML / YL
Solanaceae	*Solanum* spp. ©	Herb	D / R	1.98	NA	-
Rubiaceae	*Tricalysia* spp.	Shrub	D	1.98	LC	-

Anacardiaceae	*Anacardium occidentale* ©	Tree	D	0.99	LC	-
Rhizophoraceae	*Anisophyllea laurina*	Tree	D	0.99	LC	ML
Apocynaceae	*Cryptolepis sanguinolenta*	Climber	D	0.99	NA	-
Cucurbitaceae	*Cucumis* spp. ©	Herb	R	0.99	LC	-
Malvaceae	*Hibiscus* spp.	Herb	D	0.99	NA	-
Rubiaceae	*Mussaenda elegans*	Shrub	R	0.99	NA	-
Rubiaceae	*Rothmannia whitfieldii*	Shrub	D	0.99	LC	-

Life form: climber (C), shrub (S), tree (T). Season: dry (D) Dec-Apr; rainy (R) May-Nov. F_o(%): Frequency of occurrence, the number of times plant taxa was detected across samples (N = 207). Part eaten: flower (Fl), fruit (Fr, mostly unripe), mature leaves (ML), young leaves (YL), seeds (S); this information was obtained from previous studies (references in Table A.4.1 in Appendix 4). Status: IUCN Red List Conservation status, least concerned (LC), near threatened (NT), vulnerable (VU), not available (NA).

Table A.4.4 Complete list of plant taxa detected in the diet of Temminck's red colobus (*P. b. temminckii*) in 2009.

Characteristics of wild and crop plant taxa detected in *P. b. temminckii* non-invasive samples using a DNA metabarcoding approach.

Plant family	**Dietary taxon**	**Life form**	**Season**	**F_o(%)**	**Country status**	**Plant part eaten**
Chrysobalanaceae	*Parinari excelsa*	Tree	D	50.00	LC	Fl / Fr / ML / YL
Fabaceae	*Cassia sieberiana*	Tree / Shrub	D	38.33	LC	-
Fabaceae	*Leptoderris* spp.	Climber	D	38.33	LC	-
Fabaceae	*Mezoneuron* spp.	Vine	D	33.33	NA	-
Anacardiaceae	*Mangifera indica* ©	Tree	D	30.00	DD	-
Moraceae	*Ficus* spp.	Tree	D	28.33	LC	Fr / ML / YL
Rhizophoraceae	*Anisophyllea laurina*	Tree	D	21.67	LC	ML
Moraceae	*Milicia regia*	Tree	D	18.33	VU	-
Bombacaceae	*Ceiba pentandra*	Tree	D	15.00	LC	-
Combretaceae	*Combretum* spp.	Shrub	D	13.33	LC	Fl / ML / S / YL
Simaroubaceae	*Odyendea* spp.	Tree	D	11.67	NA	Fl / ML / S / YL
Moraceae	*Treculia africana*	Tree	D	11.67	LC	ML / YL
Meliaceae	*Trichilia prieureana*	Tree	D	11.67	LC	Fl / ML / YL
Olacaceae	*Ximenia americana*	Shrub / Tree	D	10.00	LC	-
Rubiaceae	*Mussaenda elegans*	Shrub	D	8.33	NA	-
Euphorbiaceae	*Macaranga heterophylla*	Shrub / Tree	D	6.67	LC	-
Sapindaceae	*Paullinia pinnata*	Climber	D	6.67	NA	-

Celastraceae	*Reissantia indica*	Shrub	D	6.67	NA	Fl / Fr / ML / YL
Meliaceae	*Carapa procera*	Tree	D	3.33	LC	-
Fabaceae	*Dalbergia* spp.	Tree / Shrub / Climber	D	3.33	LC	-
Convolvulaceae	*Ipomoea* spp. ©	Herb	D	3.33	LC	ML / YL
Rubiaceae	*Ixora* spp.	Shrub	D	3.33	LC	-
Rubiaceae	*Keetia venosa*	Tree / Shrub	D	3.33	LC	-
Verbenaceae	*Rinorea* spp.	Shrub	D	3.33	LC	-
Celastraceae	*Salacia* spp.	Climber	D	3.33	LC	-
Celastraceae	*Simicratea welwitschii*	Climber	D	3.33	NA	-
Rubiaceae	*Uncaria africana*	Shrub	D	3.33	NA	-
Avicenniaceae	*Avicennia germinans*	Tree / Shrub	D	1.67	LC	-
Rubiaceae	*Cremaspora triflora*	Shrub	D	1.67	LC	-
Fabaceae	*Dichrostachys cinerea*	Tree / Shrub	D	1.67	LC	Fl / ML / YL
Sapotaceae	*Malacantha alnifolia*	Shrub / Tree	D	1.67	NA	-
Rubiaceae	*Morinda lucida*	Tree / Shrub	D	1.67	LC	Fl / ML / YL
Asteraceae	*Synedrella nodiflora*	Herb	D	1.67	NA	-
Meliaceae	*Trichilia monadelpha*	Tree / Shrub	D	1.67	LC	Fl / ML / YL
Lamiaceae	*Vitex* spp.	Tree / Shrub	D	1.67	LC	Fl / YL

Life form: climber (C), shrub (S), tree (T). Season: dry (D) Dec-Apr; rainy (R) May-Nov. F_o(%): Frequency of occurrence, the number of times plant taxa was detected across samples (N = 207). Part eaten: flower (Fl), fruit (Fr, mostly unripe), mature leaves (ML), young leaves (YL), seeds (S); this information was obtained from previous studies (references in Table A.4.1 in Appendix 4). Status: IUCN Red List Conservation status, least concerned (LC), near threatened (NT), vulnerable (VU), not available (NA).

APPENDIX 4 – Supporting material for CHAPTER 4

Appendix 5 – Supporting material for CHAPTER 5

Table A.5.1 Complete list of plants consumed by primates and used by humans in Gola RNP.

Frequencies of occurrence of each wild plant in the primates diet is listed, and the plant salience for humans calculated from the freelisting results obtained during the semi-structured interviews. Darker colours indicate higher detection in the faeces, or importance to respondents (calculations presented in Chapters 3, 4 and 2). The focus of the interviews was wild plants, hence no data is available for crop species for humans.

	F_o(%)		**Salience**
Plant taxon	***P. b. badius***	***P. t. verus***	**Humans**
Abelmoschus spp. ©	0.000	66.667	na
Acridocarpus plagiopterus	1.932	0.000	0.000
Afrobrunnichia erecta	3.382	0.000	0.000
Aganope leucobotrya	1.932	0.000	0.000
Albizia zygia	0.000	0.000	0.014
Alstonia boonei	0.000	0.000	0.010
Anisophyllea laurina	0.000	0.000	0.021
Anisophyllea meniaudi	0.966	0.000	0.000
Annickia polycarpa	0.000	0.000	0.057
Anthocleista nobilis	0.000	0.000	0.007
Anthonoth macrophylla	36.715	0.000	0.000
Bambusa vulgaris	0.000	0.000	0.014
Beilschmiedia mannii	0.000	0.000	0.028
Berlinia confusa	1.932	0.000	0.000
Blighia unijugata	0.000	0.000	0.002
Brachystegia leonensis	1.449	0.000	0.000
Bridelia micrantha	7.729	0.000	0.000
Bussea occidentalis	0.000	0.000	0.026
Campylostemon spp.	0.483	0.000	0.000
Carapa procera ~	14.493	0.000	0.134
Cassia sieberiana	0.000	0.000	0.196
Chytranthus spp.	0.483	0.000	0.000
Coffea spp. ©	1.932	0.000	na
Cola lateritia	0.000	0.000	0.031
Cola spp.	2.415	0.000	0.000
Combretum spp.	33.816	0.000	0.000
Costus spp.	0.000	66.667	0.000
Craterispermum caudatum	0.483	0.000	0.000

Cryptosepalum tetraphyllum	7.246	0.000	0.000
Dactyladenia smeathmannii	1.449	0.000	0.000
Dalbergia spp.	5.797	0.000	0.000
Daniellia ogea	4.348	0.000	0.000
Dialium dinklagei	0.000	0.000	0.011
Dialium guineense	0.000	0.000	0.009
Didelotia idae	15.942	0.000	0.000
Dioscorea cayennensis	0.000	0.000	0.020
Enterolobium spp. ©	0.966	0.000	na
Eremospatha macrocarpa	0.000	0.000	0.160
Eremospatha spp.	0.000	0.000	0.288
Eugenia spp.	11.594	0.000	0.000
Ficus spp. ^	16.908	66.667	0.000
Fleroya spp.	0.483	0.000	0.000
Funtumia africana ~	0.483	0.000	0.014
Garcinia afzelii ~	0.483	0.000	0.074
Garcinia kola	0.000	0.000	0.388
Gilbertiodendron preussii	1.932	0.000	0.000
Gouania longipetala	0.000	16.667	0.000
Heckeldora leonensis	3.865	0.000	0.000
Helictonema velutinum	0.966	0.000	0.000
Hutchinsonia barbata	0.483	0.000	0.000
Hypselodelphys violacea	0.000	33.333	0.000
Ipomea pileata	0.000	0.000	0.014
Irvingia gabonensis	0.000	0.000	0.065
Keetia spp.	14.976	0.000	0.000
Laccosperma secundiflorum	0.000	0.000	0.033
Leptoderris spp.	0.483	0.000	0.000
Lophira alata	37.198	0.000	0.000
Lovoa trichilioides	0.483	0.000	0.000
Macaranga barteri *	3.382	16.667	0.022
Macaranga heterophylla	0.483	0.000	0.000
Malacantha alnifolia	0.483	0.000	0.000
Mammea africana	0.000	0.000	0.009
Manilkara obovata ^	13.527	16.667	0.000
Manniophyton fulvum	15.459	0.000	0.000
Maranthes aubrevillei	33.816	0.000	0.000
Mareya micrantha	0.000	0.000	0.010
Massularia acuminata	0.000	0.000	0.329
Megaphrynium spp.	0.000	16.667	0.000
Memecylon spp.	2.899	0.000	0.000

Morinda lucida	0.000	0.000	0.007
Morinda morindoides	0.000	0.000	0.004
Musanga cecropioides ^	0.483	100.000	0.000
Mussaenda spp.	0.483	0.000	0.000
Myrianthus arboreus ^	3.865	50.000	0.000
Napoleonaea spp.	0.966	0.000	0.000
Nauclea diderrichii	0.000	0.000	0.143
Nauclea spp.	21.256	0.000	0.000
Odyendea klaineana	0.966	0.000	0.000
Omphalocarpum pachysteloides	1.932	0.000	0.000
Paramacrolobium coeruleum	1.932	0.000	0.000
Parinari excelsa	0.000	0.000	0.034
Parinari spp.	3.382	0.000	0.000
Pentaclethra macrophylla	0.000	0.000	0.066
Pentadesma butyracea	17.391	0.000	0.000
Piper guineense	0.000	0.000	0.236
Plagiosiphon emarginatus	0.483	0.000	0.000
Platysepalum hirsutum	0.483	0.000	0.000
Protomegabaria stapfiana	0.000	0.000	0.029
Pseudospondias microcarpa	0.000	0.000	0.023
Raphia spp.	0.000	0.000	0.007
Rhigiocarya racemifera	0.483	0.000	0.000
Rhodognaphalon spp.	2.899	0.000	0.000
Ruthalicia eglandulosa	0.483	0.000	0.000
Sarcocephalus latifolius	0.000	0.000	0.077
Scleria boivinii	0.000	0.000	0.026
Sida acuta	0.000	0.000	0.016
Sterculia tragacantha	0.000	0.000	0.005
Strephonema pseudocola	11.111	0.000	0.000
Strychnos spp.	10.628	0.000	0.000
Terminalia spp.	8.213	0.000	0.000
Tetracera alnifolia	0.000	0.000	0.012
Tetracera potatoria	0.000	0.000	0.010
Tetracera spp.	12.560	0.000	0.000
Trichilia monadelpha	12.077	0.000	0.000
Uvaria chamae	0.000	0.000	0.007
Vitex spp.	0.966	0.000	0.000
Xylopia aethiopica	0.000	0.000	0.118

Plant taxa overlap between: * two primates and humans (N = 1), ~ red colobus and humans (N = 3), º chimpanzees and humans (N = 0), and ^ both primates (N = 4). © - introduced or native plant cultivated by humans and consumed by primates (N = 3: *P. b. badius*, N = 2; *P. t. verus*, N = 1).

Table A.5.2 Complete list of plants consumed by primates and used by humans in Cantanhez NP.

Frequencies of occurrence of each wild plant in the primates diet is listed, and the plant salience for humans calculated from the freelisting results obtained during the semi-structured interviews. Darker colours indicate higher detection in the faeces, or importance to respondents (calculations presented in Chapters 3, 4 and 2). The focus of the interviews was wild plants, hence no data is available for crop species for humans.

	F_o(%)		**Salience**
Plant taxon	***P. b. temminckii***	***P. t. verus***	**Humans**
Acridocarpus plagiopterus	3.960	0.000	0.000
Adansonia digitata	0.000	0.000	0.052
Aframomum spp.	0.000	0.000	0.006
Afzelia africana	0.000	0.000	0.048
Albizia adianthifolia	0.000	0.000	0.041
Albizia zygia	0.000	0.000	0.010
Allophylus africanus	0.000	0.000	0.002
Alstonia boonei	0.000	0.000	0.072
Ampelocissus leonensis	0.000	1.786	0.000
Anacardium occidentale ©	0.990	8.929	na
Anisophyllea laurina *	0.990	5.357	0.348
Annona senegalensis	0.000	0.000	0.009
Anthocleista procera	0.000	0.000	0.053
Anthostema senegalense	0.000	0.000	0.008
Antiaris toxicaria	0.000	0.000	0.029
Antidesma membranaceum	0.000	0.000	0.013
Avicennia germinans ~	18.812	0.000	0.093
Bauhinia reticulata	0.000	0.000	0.030
Bauhinia thonningii	0.000	0.000	0.011
Blighia unijugata	0.000	0.000	0.015
Borassus aethiopum	0.000	0.000	0.042
Bridelia micrantha	0.000	0.000	0.015
Caesalpinia benthamiana	0.000	0.000	0.010
Calamus deerratus	0.000	0.000	0.021
Calycobolus heudelotii	0.000	0.000	0.025
Carapa procera ~	1.980	0.000	0.007
Cassia sieberiana ~	24.752	0.000	0.255
Ceiba pentandra *	15.842	44.643	0.080
Citrus limon	0.000	1.786	0.000
Coffea spp.	3.960	0.000	0.000

Combretum micranthum	0.000	0.000	0.074
Combretum spp.	26.733	0.000	0.000
Craterispermum laurinum	0.000	0.000	0.012
Cremaspora triflora	1.980	0.000	0.000
Crudia senegalensis	8.911	0.000	0.000
Cryptolepis sanguinolenta	0.990	0.000	0.000
Cucumis spp. ©	0.990	0.000	na
Cyperaceae (Fam.)	0.000	0.000	0.002
Dalbergia spp. ^	1.980	5.357	0.000
Daniellia oliveri	0.000	0.000	0.018
Detarium microcarpum	0.000	0.000	0.016
Detarium senegalense	0.000	0.000	0.003
Dialium guineense	0.000	0.000	0.361
Dichrostachys cinerea ~	12.871	0.000	0.006
Elaeis guineensis	0.000	0.000	0.254
Erythrina senegalensis	0.000	0.000	0.004
Ficus exasperata	0.000	0.000	0.012
Ficus spp. *	38.614	78.571	0.009
Ficus sur	0.000	0.000	0.042
Harungana madagascariensis	17.822	0.000	0.000
Hexalobus monopetalus	0.000	0.000	0.002
Hibiscus spp.	0.990	0.000	0.000
Holarrhena floribunda	0.000	0.000	0.015
Hunteria umbelata	0.000	0.000	0.071
Hymenocardia acida	0.000	0.000	0.004
Imperata cylindrica	0.000	0.000	0.003
Ixora spp.	25.743	0.000	0.000
Keetia venosa	5.941	0.000	0.000
Khaya senegalensis	0.000	0.000	0.113
Klainedoxa gabonensis	0.000	0.000	0.008
Laguncularia racemosa	0.000	0.000	0.011
Landolphia heudelotii	0.000	0.000	0.048
Landolphia spp.	0.000	0.000	0.083
Lannea acida	0.000	0.000	0.011
Leptoderris spp.	39.604	0.000	0.000
Lophira lanceolata	0.000	0.000	0.004
Macaranga heterophylla	3.960	0.000	0.000
Malacantha alnifolia	34.653	0.000	0.000
Mangifera indica ©	8.911	23.214	na
Merremia spp.	0.000	1.786	0.000
Mezoneuron benthamianum ^	14.851	1.786	0.000

Milicia regia ^	12.871	23.214	0.000
Mimusops adongensis ^	2.970	5.357	0.000
Morinda chrysorhiza	0.000	0.000	0.013
Mussaenda elegans ^	0.990	7.143	0.000
Neocarya macrophylla *	1.980	16.071	0.058
Newbouldia laevis	0.000	0.000	0.010
Odyendea klaineana ^	2.970	1.786	0.000
Oxytenanthera abyssinica	0.000	0.000	0.018
Parinari excelsa *	39.604	10.714	0.324
Parkia biglobosa	0.000	0.000	0.077
Paullinia pinnata	3.960	0.000	0.000
Pentaclethra macrophylla	0.000	0.000	0.001
Phyllanthus muellerianus *	12.871	19.643	0.006
Psychotria peduncularis	0.000	0.000	0.019
Pterocarpus erinaceus	0.000	0.000	0.158
Pterocarpus santalinoides	0.000	0.000	0.003
Pterocarpus spp.	1.980	0.000	0.000
Raphia palma-pinus	0.000	0.000	0.011
Reissantia indica	1.980	0.000	0.000
Rothmannia whitfieldii	0.990	0.000	0.000
Saba senegalensis	0.000	0.000	0.044
Sarcocephalus latifolius	0.000	0.000	0.100
Schoenoplectus litoralis	0.000	0.000	0.008
Schrebera arborea	0.000	0.000	0.037
Securidaca longipedunculata	0.000	0.000	0.025
Senna podocarpa	0.000	0.000	0.013
Simicratea welwitschii	13.861	0.000	0.000
Solanum spp. ©	1.980	0.000	na
Sorindeia juglandifolia	0.000	0.000	0.017
Spondias mombin	0.000	0.000	0.101
Sterculia tragacantha	0.000	0.000	0.018
Strombosia pustulata	0.000	0.000	0.045
Strophanthus hispidus	0.000	0.000	0.058
Strophanthus sarmentosus	0.000	0.000	0.023
Terminalia macroptera	0.000	0.000	0.004
Tetracera potatoria ^	22.772	1.786	0.000
Treculia africana ^	51.485	23.214	0.000
Trema orientalis °	0.000	3.571	0.046
Tricalysia spp.	1.980	0.000	0.000
Trichilia monadelpha	5.941	0.000	0.000
Trichilia prieureana ^	12.871	1.786	0.000

Triumfetta spp.	0.000	3.571	0.000
Uncaria africana	16.832	0.000	0.000
Urera oblongifolia	0.000	3.571	0.000
Uvaria chamae	0.000	0.000	0.194
Vigna spp.	0.000	7.143	0.000
Vitex doniana	0.000	0.000	0.031
Voacanga africana	0.000	0.000	0.015
Ximenia americana	4.950	0.000	0.000
Xylopia aethiopica	0.000	0.000	0.080
Zanthoxylum leprieurii	0.000	0.000	0.021
Zanthoxylum zanthoxyloides	0.000	0.000	0.066

Plant taxa overlap between: * two primates and humans (N = 6), ~ red colobus and humans (N = 4), º chimpanzees and humans (N = 1), and ^ both primates (N = 9). © - introduced or native plant cultivated by humans and consumed by primates (N = 4: both primate, N = 2; *P. b. badius*, N = 2; *P. t. verus*, N = 0).

Figure A.5.1 Intra-species variation in diet of primates in Gola RNP.

Diet histograms at the park level and per primate group (transect) for western red colobus (left column) and western chimpanzees (right column), illustrating differences in total niche breadth (rightmost bar on top graphs) and groups diet variation between the two primates. Groups refer to samples collected in the same transect, and they are located in Gola Central (T34, T37, T40, T44, T47, T55) and Gola South (T1C, T08, T10, CF).

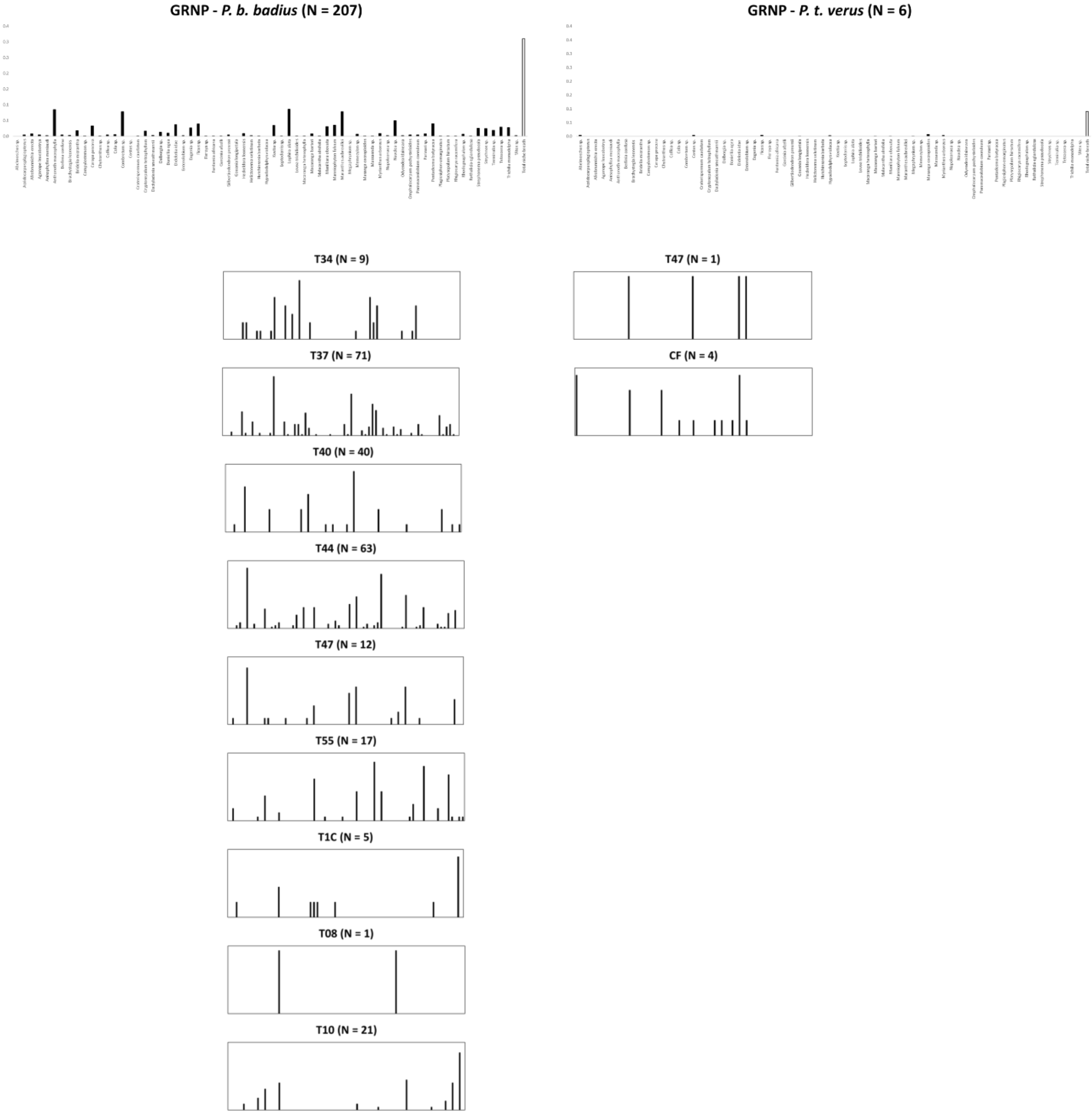
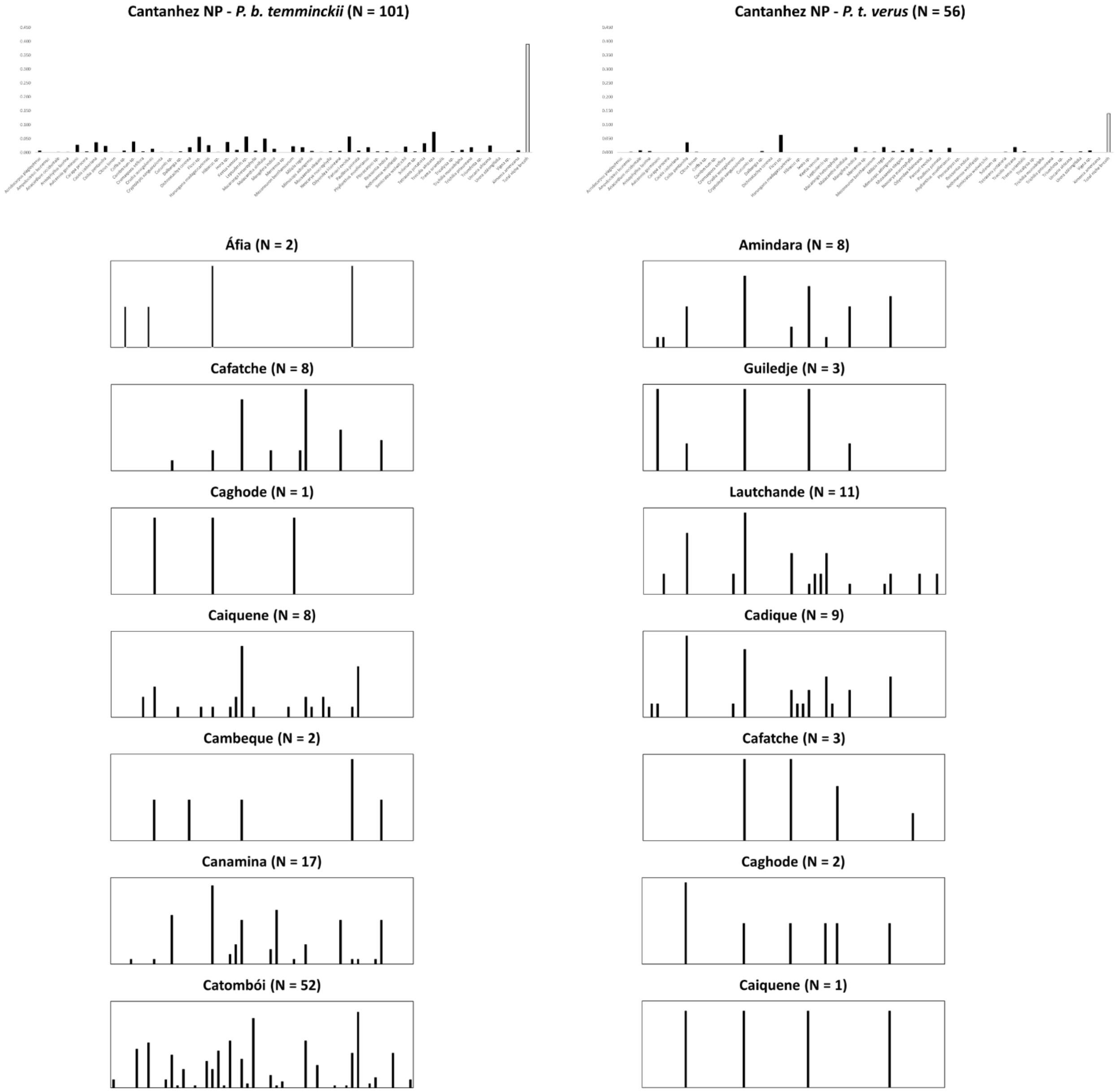
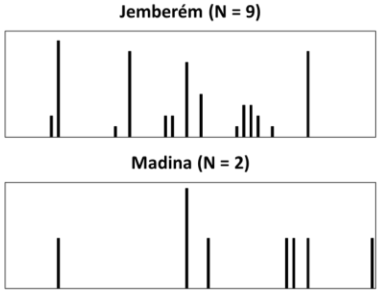


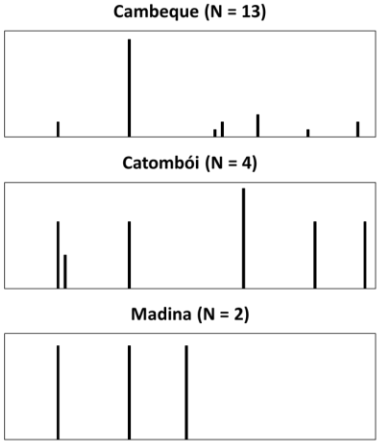
Figure A.5.2 Intra-species variation in diet of primates in Cantanhez NP.

Diet histograms at the park level and primate group (forest fragment) for western red colobus (left column) and western chimpanzees (right column), illustrating differences in total niche breadth (rightmost bar on top graphs) and groups diet variation between the two primates. Groups refer to samples collected in the same forest fragment, and they are located in the north of the park where savanna is predominant (Amindara, Áfia, Guiledje, Lautchande) and the south region which is mainly forest and mangroves (Cadique, Caiquene, Cafatche, Caghode, Canamina, Catombói, Madina, Cambqeue, Jemberém).




Jemberém (N = 9)

Madina (N = 2)


Cambeque (N = 13)

Catombói (N = 4)

Madina (N = 2)

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