Morphological and genetic variation in samango monkeys (*Cercopithecus albogularis*) in southern Africa

NTUTHUZELO MAKHASI

Dissertation submitted in fulfilment of the requirements for the Master of Science (MSc) degree in the Faculty of Agriculture and Science, School of Biological and Environmental Sciences, Department of Zoology and Entomology, University of Fort Hare, Alice Campus, EC, South Africa.

Supervisor: Prof. Judith Masters

Co-supervisor: Dr Fabien Génin

28 March 2013

Frontispiece. A pair of *Cercopithecus albogularis labiatus* from Hogsback, Eastern Cape. Photo by Ken Harvey

DECLARATION

The work described in this dissertation was carried out in the School of Biological and Environmental Sciences, University of Fort Hare, main campus, Alice, from January 2010 to March 2013, under the supervision of Professor Judith Masters and Dr Fabien Génin.

I, Ntuthuzelo Makhasi, declare that the research work reported in this dissertation, except where otherwise stated, is my original work. In addition to that this thesis was not submitted for any degree or diploma at any other university.

Therefore, this thesis does not contain another person's data, pictures, graphs or other information, unless otherwise stated and referenced. Furthermore and lastly, in cases where exact words of others have been re-written, such writing will be placed in italics inside quotation marks and referenced at the end.

Signed: Ntuthuzelo Makhasi

At: Department of Zoology and Entomology

Date: 28 March 2013

ACKNOWLEDGEMENTS

First, I would like to express my gratitude to my supervisors, Prof. Judith Masters and Dr Fabien Génin for giving me the responsibility and opportunity to execute this research with their consistent guidance, assistance and incredible support throughout the project. In addition, I thank Prof. Paul Grobler and Riel Coetzer of the University of Free State, Department of Genetics, and Dr Solomon Tesfamichael, Department of Geography, University of Fort Hare (UFH), for their crucial contribution in this research.

Secondly, I am grateful for the financial support I received from the National Research Foundation (NRF) and the University. I am grateful to UFH for academic assistance and to the UFH Animal Ethics Committee for approval of the sampling methods. I thank the museum curators who generously made their collections available to me: Mr. Lucas Thibedi at Amathole Museum in King William's Town, Eastern Cape; Dr. Teresa Kearney at the Transvaal Museum (now Ditsong Museum) in Pretoria, Gauteng Province; Prof. Peter Taylor at Durban Natural Science Museum in Durban, KwaZulu-Natal; and Kith Mkwanazi at the Natural History Museum of Zimbabwe in Bulawayo, Zimbabwe.

I thank Ken Harvey, the nature photographer in Hogsback for providing the frontispiece for this dissertation. Dr Mathew Blatchford and Prof. Felicity Wood were a living inspiration to me each time we shared a dinner table in Hogsback. Special thanks to my lovely and loving girlfriend, Noma J. Sokhuthu for her sacrifices and amazing support throughout this project, and to my brother Sibusiso Makhasi; my sister Nomava Mangaliso and the rest of Makhasi and Mangaliso family for their motivation. Lastly, I thank Zanoxolo Mbeka, J. C. van der Walt and Vuyo Gladstone Nongqo (my former English High School teacher) for their various inputs into this research. I thank the Almighty for all of the above and for the gift of life to have such a wonderful opportunity.

ABSTRACT

My aim was to resolve the taxonomy of the South African forms of *Cercopithecus albogularis* by exploring morphological and genetic variation in the two samango subspecies described for the region: *C. a. erythrarchus* and *C. a. labiatus*. In addition, I estimated their geographic distributions and habitat requirements from the provenance data I collected during my study of museum specimens. My analysis has shown clear morphological differences between *C. a. labiatus* and *C. a. erythrarchus*. The two subspecies differ in pelage coloration, but also in cranial shape and tail lengths. Furthermore, *C. a. labiatus* is slightly smaller, shorter-tailed and stocky compared with the long-tailed slender northern forms of *C. albogularis*, which may be adaptations to cold environments like montane forest. The northern *C. albogularis* subspecies are distinguishable from the southern taxa with a high degree of reliability (98.5%), suggesting that *C. albogularis* consists of more than one species. The most appropriate name for the southern species is *C. labiatus.* My genetic study did not detect variation among the 10 animals sampled in Hogsback; while it clustered the different taxa, it could not resolve relationships between them, with the exception of the outgroup. The lack of resolution of the deeper nodes could be a result of the fact that our sequence was very short (274 bp). The mt 12S rRNA gene was not an ideal gene for this study, which should have involved a less conserved section of the mtDNA molecule, like the rapidly evolving D-loop. More genetic work is clearly needed to resolve the phylogenetic relationships within the *C. mitis* supergroup. However, preliminary genetic data indicate that the southern samangos are distinct from the *C. mitis* of West Africa, while my morphometric study suggests they may also be distinct from *C. albogularis* in East Africa. Molecular and karyological studies comparing the genomes of Hogsback "*C. a. labiatus*" with the neighbouring "*C. a. erythrarchus*" and Zanzibar *C. albogularis* would be extremely enlightening.

CONTENTS

Chapter 3: Genetic Differentiation

Chapter 5: Discussion and Conclusions

Appendices

LIST OF FIGURES

LIST OF TABLES

Chapter One: Biodiversity

1.1. Introduction

1.1.1. South African biodiversity

South Africa is one of 17 megadiverse countries which collectively contain more than two-thirds of global biodiversity, based on an index of species diversity and endemism (http://en.wikipedia.org/wiki/Megadiverse_countries, downloaded 25 March 2013). South Africa's faunal diversity is relatively high compared with its land surface area; covering only 2% of the global land area, South Africa is home to an estimated 6% of the global total of mammal species (close to 260 species), and ranks as the fifth richest country in Africa and the 24th richest in the world in terms of biodiversity (Groombridge, 1992).

South Africa has a wide range of climatic conditions and highly variable topography (e.g. narrow coastal plain, steep escarpment, large plateau). In combination, climate and topography give rise to broad vegetation zones which, together with their associated animal life, are called biomes. These are the karoo, fynbos, forest, grassland and savanna biomes. Each of these supports its own collection of plant and animal species. The karoo, for example, is home to plants and animals well suited to hot, dry conditions such as gemsbok, and succulent plants. The Fynbos flora is extremely rich in species that are adapted to a Mediterranean climate and the poor soils of the south-western Cape.

South Africa has a total land surface area of 1 219 912 km^2 and the national territory includes the sub-Atlantic Marion and Prince Edward Islands (Friedman and Daly, 2006). It is host to a wide variety of ecosystems. Agriculture makes up the major land use, and contributes about 3.2% to the Gross Domestic Product. Urban settlements comprise

approximately 1.14% of the land. There are currently about 51 million people living in South Africa (Statistics South Africa, Census 2011).

An early assessment of the conservation status of South African mammals was published by Smithers (1986), but included terrestrial mammals only. The most comprehensive assessment of the conservation status of South Africa's mammalian species was undertaken in 2002/2003. The *Red Data Book of Mammals of South Africa* (Friedman and Daly, 2006) indicated that, of the 295 species and subspecies of South African mammals evaluated, 12% were endemic, 57 (19.3%) were considered threatened (critically endangered, endangered or vulnerable), while a further 38 (12.8%) were near threatened; only 147 (49.8%) were regarded as of least concern. Of the critically endangered and endangered species, 70% and 33%, respectively, were endemic to South Africa. Almost a fifth, 53 (18%) of all mammal species could not be assessed due to data deficiencies. The major threats to terrestrial mammals are habitat loss and land transformation due to deforestation, agriculture, timber planting and urban and industrial development, as well as poisoning, pollution and hunting.

1.1.2. Defining biodiversity

Biodiversity refers to the variety of life forms at a particular level of biological organization, from all sources, including terrestrial, marine and other aquatic ecosystems and ecological components which comprise them (Gaston and Spicer, 2004). This includes diversity within species, between species and among ecosystems. The diversity of life can be registered in various ways in terms of key elements comprising three nested hierarchies of genetic, organismal and ecological diversity. Genetic diversity includes elements of the genetic coding that structures organisms, (e.g. nucleotides, genes and chromosomes) and variation in the genetic make-up between individuals within a population and between populations.

Organismal diversity includes taxonomic nested hierarchies (e.g. domains or kingdoms, phyla, families, orders, classes, genera, species, subspecies, populations and individuals). Lastly, ecological diversity categorizes variation among ecosystems, (e.g. populations, niches, habitats, ecosystems, landscapes, bioregions and biomes). Though it may be difficult to define the limits of elements of biodiversity (such as species or populations), they remain useful and important concepts for thinking about and studying biodiversity. The genetic, organismal and ecological hierarchies of biodiversity are inter-dependent and share elements in common, e.g. populations. Their nestedness allows us to trace the complexity of biodiversity; for example, within genetic diversity, populations are constituted of individuals, each individual has a complement of chromosomes, these chromosomes comprise numbers of genes, and genes are constructed from nucleotides. Likewise, within organismal diversity kingdoms, phyla, families, genera, species, subspecies, populations and individuals form a nested sequence, in which all elements at lower levels belong to one example of each of the elements at higher levels.

1.1.3. Measuring biodiversity

In order to assess how biodiversity has changed through time, to understand where it occurs, and how it can be maintained, it is important to quantify it in some way. However, given its complexity, it cannot be expressed as a single measure, and there are numerous ways in which differences can be shown. In practice species richness, i.e. the number of endemic species per unit area tends to be the most commonly used measure, because it is practically possible for different researchers to provide similar, repeatable results of the number of species of a particular status (e.g. present, breeding, etc.) in a given taxon in a certain area at a given time. A great deal of information already exists on patterns in species richness and is made available in the scientific literature, and museum collections can be used to gather information on species richness over historical time. The species unit is commonly seen as

the unit of practical management, legislation, politics, and tradition. Variation in biodiversity is seen as variation in species numbers by many people (Gaston and Spicer, 2004).

There are, however, some limitations, one of which is the on-going disagreement among systematists as to how to define a species. Some researchers (e.g. Groves, 2001) regard species as hypotheses, opinions or concepts, rather than objectively real entities. I have investigated seven major species concepts, each with its strengths and weaknesses, from either theoretical or practical perspectives. The application of these different concepts can lead to the recognition of different numbers of species. Because recognising appropriate units of biodiversity is crucial for understanding species richness, which in turn is the most common tool for studying biodiversity (Gaston and Spicer, 2004), I have chosen to focus on the Recognition Concept (Paterson, 1985), which has been used effectively to study species diversity among living primates (Zimmermann, 1990; Masters, 1991, 1993; Bearder *et al.*, 1995; Zimmermann *et al.*, 1998). The Recognition Concept defines species as populations that have systems of communication and signal exchange that allow them to recognize conspecific mating partners. I chose this species concept because cercopithecoid monkeys are largely diurnal, and they communicate chiefly using visual and auditory signals; the similarity of their sensory systems makes their signals particularly accessible to humans.

1.1.4. The species concept

Models of species and their origins are still disputed among evolutionary biologists (Masters, 1998). Evolutionary processes are divided into two hierarchical categories, namely, microevolution and macroevolution. Microevolution describes evolutionary changes at the population level, while macroevolution describes changes at and above the species level (Grantham 2007). Adaptation is an example of microevolution, while speciation, the formation of a new species from a parent species, is the interface between the two categories.

Some authors consider speciation as a resultant effect of adaptation (Hansen and Martins, 1996; Irwin *et al.*, 2001; Grantham, 2007), while others (e.g. Masters and Rayner 1993; Masters, 1998; McNamara and McKinney, 2005) see it as involving more than just a summation of gradual changes, and possibly even genomic re-organization. Speciation has been a controversial subject in evolutionary biology because of the confusion of speciation and adaptation since Darwin's publication of "*Origin*" (Masters, 1998; Masters, 2000). The study of speciation cannot be separated from the species concept in use. Speciation is an irreversible event that may be morphologically cryptic. It is not difficult to distinguish between a chimp and a human, but morphology is not always so species-specific, as in the case of nocturnal primate species (Masters, 1993).

The use of reproduction/gene exchange as a way of defining species was introduced in the 18th Century, and was incorporated into the New Synthesis in the guise of the Biological Species Concept, which became very popular in the 20th Century. Paterson (1978; 1985) proposed the Recognition Concept (RC) of species as an alternative view of gene exchange: "the most inclusive population of biparental organisms which share a common fertilisation system". According to the RC, a species is self-defining, not defined in relation to other species. In mobile sexual organisms, a major component of the fertilisation system is the Specific-Mate Recognition System (SMRS). The SMRS is comprised of communication signals (odours, calls and visual signals) and courtship behaviours.

Whereas some species concepts treat speciation as a simple extrapolation of adaptation and/or drift, the RC is more explicit about the operation and effect of these processes. During an ecological shift due to habitat loss or degradation, natural selection drives adaption to the new environment, not necessarily speciation. New species are formed only if the environmental changes disturb the effectiveness of the SMRS. The RC predicts

that the SMRS should be relatively stable in time and space, and only be affected by major environmental disruptions.

Identification and formation of species are important to evolutionary and conservation biology. Inter- and intra-specific comparisons are impossible without a reliable species definition. Unrealistic definitions can cause widespread confusion, e.g. taxonomic inflation. Such inflation may underestimate or overestimate the conservation priority of some taxa, misdirecting conservation efforts and funding (Agapow *et al.*, 2004). From the RC point of view, species are real and stable entities. Individuals or populations of a species share the same SMRS, and speciation occurs when environmental changes disrupt the SMRS. Therefore, speciation occurs only in allopatry with substantial ecological shifts (Masters, 1993; Masters, 1998; Masters, 2000).

The RC predictions are as follows: 1) SMRS characters are species-specific and environment-specific. 2) Speciation occurs when the SMRS is no longer effective in the new environment. 3) Because of the stability of the SMRS, speciation is always accompanied by niche shifts. 4) The new species should not have the same ecological niche as its parent species. Samangos can be used to explore this speciation model because of their extensive and recent radiation and, sometimes, sympatric distributions.

1.1.5. Species vs. *subspecies*

Some species have well-defined geographic variation and may readily be divided into subspecies; they are said to be polytypic. By definition, subspecies do not inhabit the same region. The subspecies concept, with its trinomial nomenclature, was established during the latter half of the 19th century. It is useful in conservation to describe and characterize population groups in which the majority of individuals differ from other such groups. However, subspecies are not often recognized by conservation authorities.

My study focuses on the intraspecific variation in South African samango monkeys, where two subspecies have been recognized: *Cercopithecus albogularis labiatus* and *C. a. erythrarchus*. No previous study has investigated the differences between these two populations in any detail. *C. a. labiatus* is listed in the *Red Data Book of Mammals of South Africa* (Friedman and Daly, 2006) as endangered and is the country's only endangered primate taxon. This fact makes my study particularly important to our understanding of our national biodiversity.

1.1.6. Taxonomy and conservation biology

In order to work meaningfully with biodiversity, it is necessary to place organisms in groups, or taxa, which reflect their evolutionary relationships. The largest grouping is the kingdom, within which, in decreasing order of inclusiveness, are the phylum, class, order, family, genus and species. Working taxonomists have also introduced additional subdivisions such as subphylum, suborder, superclass, etc., if the main hierarchal levels are too few to convey natural relationships adequately. Taxonomists frequently disagree about groupings and heated arguments can occur. The naming and description of species was very haphazard until the invention of binomial nomenclature by Carl von Linné (Linnaeus) in the middle of the 18th century. The Linnaean system of naming and classifying species was almost universally adopted and remains in use, although it has been challenged recently by some biodiversity scientists (e.g. the Phylocode, see Benton, 2000). It is logical, clear and precise. Disputes concerning nomenclature are settled by the International Committee on Zoological Nomenclature. I use traditional Linnean nomenclature in my discussion of samango monkeys.

1.1.7. The species identity of the South African samango monkeys

According to Napier (1981), Pocock (1907) recognized two species groups pertaining to the *C. mitis* supergroup: *C. leucampyx* and *C. albogularis*. Schwarz (1928), who was well-known as a taxonomic "lumper", united them in a single species under the name *C. leucampyx* but, in 1933, he noted that the name *C. mitis*, which had been diagnosed by Wolf in 1822, had priority. In 1966, Hill restored the two taxa to specific status, together with *C. nictitans* within his superspecies, *C. mitis* (Napier, 1981). The inclusion of *C. nictitans* within the *C. mitis* species group was also indicated by a recent molecular study (Tosi *et al*., 2005).

The species name of South African samango monkeys is still disputed. Some researchers (e.g. Lawes, 1990, 1992; Kingdon *et al.*, 2008) referred these populations to *Cercopithecus mitis*, while others (e.g. Meester and Setzer, 1971; Meester *et al.*, 1986) classified them as *C. albogularis*. Sineo (1990) supported the species allocation of Meester and colleagues on the basis of their karyotypic structure. In deference to Sineo's preliminary genetic study of this group, and in accordance with the taxonomy of Groves (2001), I will follow this taxonomic assignment. Samango monkeys are also known as Sykes' monkey, after their describer, or blue monkeys to distinguish them "green monkeys" or vervets.

Cercopithecus albogularis Sykes, 1831 is distributed "[f]rom Somalia southward, east of the rift valley and the Luanga Valley, to the Eastern Cape" (Napier, 1981). In Kenya, *C. albogularis* and *C. mitis* are separated by the unsuitable habitat in the Rift Valley. Further south, there are apparent hybrids in Ngorongoro and at Lake Manyara; for this reason Booth (1968) united them into a single species, and in this he was followed by Napier (1981) and others. This is still the only region from which hybrids are known.

In Tables 1.1 to 1.4 I summarize the systematic and morphological characteristics of *C. albogularis* populations in Africa, and the history of their nomenclature.

Table 1.1. General characteristics of the *C. albogularis* group

Head and body	**Limbs**	**Underparts**	**Tail**	**Significant marking (rostrally)**	**Significant marking (caudally)**
Dark grey, yellow or reddish (ticked or speckled with black); sometimes with contrasting black crown and shoulders	Arms, hands and feet black; legs black or ticked greyish	Black, grey or whitish	Like back with terminal portion black	Diadem of stiff speckled hairs, bushy speckled cheek whiskers form a ruff; white collar in some East African groups	Red or red-brown patches or tufts at base of the tail in some groups

Table 1.2. *Cercopithecus albogularis* subspecies, authors, type localities and common names, as recognized by Napier (1981)

Subspecies and Author	**Type locality**	**Common name**
C. a. albogularis (Sykes, 1831)	Zanzibar Island; this is the type locality for the species, and Schwarz (1927b) restricted this subspecies epithet to animals found on Zanzibar.	Sykes' monkey
C. a. erythrarchus (Peters,1852)	Mozambique.	Mozambique monkey/Stairs' monkey
C. a. labiatus (I. Geoffroy, 1843)	KwaZulu-Natal, South Africa.	Samango monkey (from the Xhosa name *intsimango*).

Table 1.3. Nomenclature related to *Cercopithecus albogularis erythrarchus* Peters, 1852

Year	**Species**	**Author**	**Locality**
1852	*Cercopithecus erythrarchus*	Peters	Mozambique, Inhambane
1892	*Cercopithecus stairsi*	Sclater	Mozambique, Chinde, Zambesi delta
1907	*Cercopithecus albogularis beirensis*	Pocock	Mozambique, Beira
1907	*Cercopithecus stairsi mossambicus*	Pocock	Mozambique.
1928	*Cercopithecus leucampyx nyasae*	Schwarz	Malawi Mlanje, Fort Lister
1948	*Cercopithecus mitis stevensi*	Roberts	Zimbabwe, Mount Selinda, Melsetter

Table 1.4. Nomenclature related to *Cercopithecus albogularis labiatus* I. Geoffroy, 1842

Year	**Species**	**Author**	**Locality**
1842	*Cercopithecus labiatus*	I. Geoffroy	South Africa
1845	*Cercopithecus samango*	Wahlberg	South Africa: inland of Port Natal (= Durban).
1853	*Cercopithecus chimango*	Temminck	South Africa

1.1.8. The South African subspecies of Cercopithecus albogularis

Meester *et al*. (1986) and Skinner and Chimimba (2005) listed two subspecies from the sub-Saharan region: *Cercopithecus albogularis labiatus* I. Geoffroy Saint-Hilaire, 1843 (found in Eastern Cape and KwaZulu-Natal highlands and southern Mpumalanga) and *C. a. erythrarchus* Peters, 1852 (distributed from northern KwaZulu-Natal, Limpopo, eastern Zimbabwe and Mozambique). Butynski *et al.* (2013), in the most recent review of the systematic diversity of African primates, considers members of the *C. albogularis* species group as subspecies of "*C. mitis*", and the distributions of these populations is illustrated in Fig. 1.1.

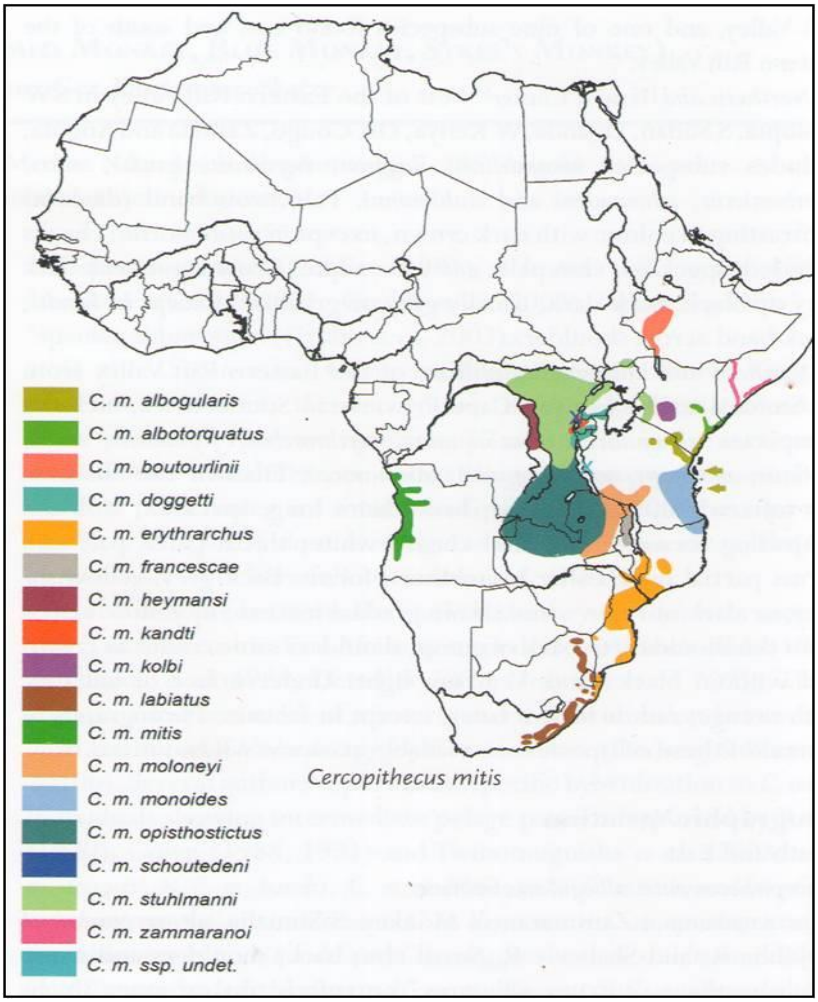

Fig 1.1. Distributions of subspecies of *Cercopithecus mitis*, from Butynski *et al.* (2013)

These are the only South African subspecies recognized in my thesis (Fig. 1.2, Table 1.5).

(a) **(b)**

Fig.1.2. (a) *C. a. erythrarchus* and (b) *C. a. labiatus*

The two subspecies occur in habitats with different structures and component species. Although the distribution ranges of the two subspecies seem large, patches of suitable habitat are not (Lawes, 1990). *C. a. erythrarchus* inhabits a variety of forest types, from coastal lowland forest and thicket, to riverine, swamp, deciduous dry and coastal dune forest (Lawes, 1992). These animals are relatively common in the coastal reserves of Ezemvelo KZN Wildlife, especially Sodwana Bay and Cape Vidal, and are not considered to be at risk. In contrast, *C. a. labiatus* occurs chiefly in Afromontane forests. These habitats are, by definition, restricted areas, patchily distributed across the eastern part of the country wherever the altitude and climate combine to support Afromontane vegetation. This means the *C. a.*

labiatus populations will be similarly restricted in size, and fragmented by vast tracts of inhospitable habitat (Lawes, 1990). In recent years, this fragmentation has been worsened by the fact that logging and habitat destruction is common in Afromontane areas (Kingdon *et al*., 2008). Lawes (1992) believes that samango populations in some of the Afromontane areas of KwaZulu-Natal are already in irreversible decline. Table 1.5 summarizes the morphological differences between *C. a. erythrarchus* and *C. a. labiatus.*

Table 1.5. The characteristics distinguishing the South African subspecies of *Cercopithecus albogularis*

Subspecies and range	**Diadem and whiskers**	**Throat patch and collar**	**Crown and nape**	**Shoulders and back**	**Colour contrast beneath tail**	**Habitat preference**
C. a. erythrarchus (central Mozambique and N.E of Zimbabwe, Limpopo Province and northern KZN)	Yellowish-grey (yellow speckling)	Throat patch whitish; collar absent	Darker than whiskers; red patches on sides of crown in erythristic forms (i.e. individuals with red patches)	Yellowish-grey (buff-speckling) back grey-brown or reddish	Red hairs laterally and caudally; white medially and ventrally	Inhabits a variety of forests, i.e. coastal lowland, and thicket, to riverine, swamp, deciduous dry and coastal dune forest
C. a. labiatus (Eastern Cape to KZN)	Yellowish-grey (buff speckling); diadem darker	Throat patch whitish, extending around neck to form an incipient collar in some)	Like diadem (darker) than whiskers; white moustache in some but variable	Dark grey (buff ticking) slightly yellower posteriorly	Long cream-white hairs	Occurs only in Afromontane forests

If *C. a. labiatus* and *C. a. erythrachus* are not just subspecific variants (i.e. adapted to slightly different habitat conditions) but distinct species, then their gene pools are separate and their adaptive and survival capacities are different. This information would be crucial to any conservation planning involving these taxa. It is in the light of this possibility that I undertook to investigate the morphological and potentially genetic diversity of the South African samango populations.

1.1.9. Conservation status

Taxonomic uncertainty clouds our understanding of biology, and this in turn impedes our ability to conserve species under threat of extinction, as species are the subject of conservation planning, not subspecies or populations (Myers *et al.*, 2000). The classification of the southern populations of samango monkeys under the widely distributed taxon superspecies *Cercopithecus albogularis* has led them to be regarded as "of least concern" by conservation authorities like the IUCN Red List, and thus not a priority for conservation (Kingdon *et al*., 2008; Butynski *et al.*, 2013). However, if the southern African populations are ecologically distinct, or even specifically distinct, from those further north in Africa, this classification obscures their true conservation status. Several subspecies are listed by the IUCN/SCC Primate Specialist Group as endangered and critically endangered (www.primate-sg.org). Indeed, *C. a. labiatus* is listed as "endangered" in the *Red Data Book of the Mammals of South Africa: a Conservation Assessment* (Friedman and Daly 2006). My study contributes to resolving the taxonomy of the *Cercopithecus albogularis* species group by exploring the morphological and genetic variation in the two South African *C. albogularis* subspecies, *C. a. erythrarchus* and *C. a. labiatus*. Subspecies need to be conserved to protect the loss of genetic diversity in the species, as this diversity is an essential part of the ability of organisms to survive climatic and vegetational changes (Gaston and Spicer, 2004).

1.2. Aim of the study

I aim to clarify the taxonomy of the South African forms of *Cercopithecus albogularis* by exploring morphological and genetic variation in the two samango subspecies described for the region: *C. a. erythrarchus* and *C. a. labiatus*. In addition, I will explore their geographic distributions and habitat requirements from the limited provenance data I have collected during my study of museum specimens.

Chapter Two: Morphological Variation

2.1 Introduction

2.1.1. Morphological variation within and between guenon species

Analyses of variation in coat colour, body size, and craniofacial morphology have all been applied to the task of understanding relationships among primate populations (Hershkovitz, 1977; Moore and Cheverud, 1992; Ferrari, 1993; Cheverud, 1995, 1996). Guenon species have striking pelage patterns that show marked interspecific variation, although their skulls and skeletons are strongly similar from species to species. For instance, *C. albogularis* are blue-grey in colour with blackish limbs (especially arms) and a broad, dark band joining them across scapular region (Napier, 1981; see also frontispiece). The nape and crown are dark grey or black, and they have pale hair on the chin and on the diadem in front (i.e. tufted eyebrows). The tail is longer than the combined head-body length in all species (Napier, 1981) and adult males (7-9 kg) are larger than adult females (4-5 kg).

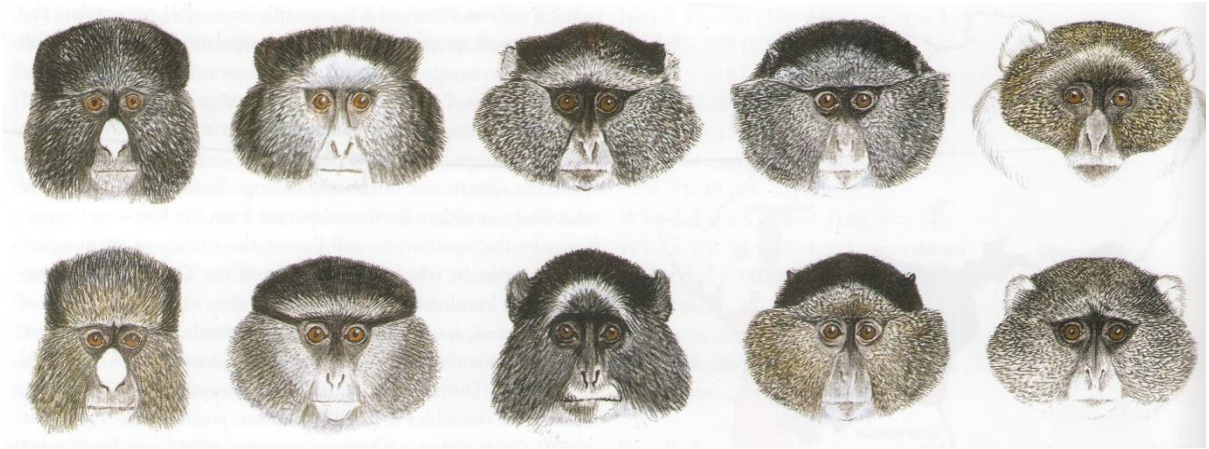

Fig. 2.1. Guenon faces (from Kingdon, 1997; reprinted in Butynski *et al.*, 2013)

Morphology may be used in classification at the species and subspecies level. Several species of guenons demonstrate considerable geographical variation in pelage colouration, particularly of the face, and this has often formed the premise for delineation of taxa (Fig.

2.1). They also show colourful differences in the rump and scrotal region (Kingdon, 1997; Butynski *et al.*, 2013). Thus guenon morphology has led to a multiplicity of described species and systematic arrangements.

Patterns of morphological variation within species play an important role in determining patterns of evolutionary diversification; evolutionary change depends on intraspecific variation as the fuel for population diversification (Lande, 1979). If subspecies of *C. albogularis* diverged via genetic drift, then the variation pattern between populations (or subspecies) would be proportional to the morphological variation within them. Under a model of genetic drift, it is expected that more variable traits will show greater amounts of drift than less variable traits. In contrast, if populations have diverged under selection, between- and within-group variation patterns will not be so predictable, and only certain traits will show adaptive differentiation (Lande, 1979). My aim is to provide insight into the processes of population evolution within *C. albogularis* in South Africa, by comparing the morphological variation in the two subspecies in relation to their geographic distributions, and using my results to infer aspects of their evolutionary history.

The identification of subspecies is essential for the research and conservation of these taxonomic units at appropriate geographical scales. A proper understanding of species status is a crucial tool in biodiversity studies, and is particularly important in the case of threatened or endangered populations. Subspecies or morphologically similar species (i.e. cryptic or sibling species) may have very different resource requirements and ecological tolerances. If *C. a. erythrarchus* and *C. a. labiatus* are considered as trivial variants of a single homogeneous group, they are regarded as "of least concern" by conservation authorities. However, only *C. a. erythrarchus*, with its wider habitat tolerance, is truly "of least concern". *C.a. labiatus*, the subspecies found in Afromotane forests, is apparently in decline throughout its distribution, and requires a different conservation strategy from its close relative. It was

listed as "endangered" in the *Red Data Book of the Mammals of South Africa: A Conservation Assessment* (Friedman and Daly, 2006), primarily because it is largely restricted to isolated fragments of Afromontane forest, which are encroached upon by logging and other agricultural activities, putting them under threat of degradation (Kingdon *et al*. 2008).

Morphological differences in relative cranial proportions could indicate differences in adaptation and thus could provide insights into the ecology of the two subspecies. Differences in adaptation may provide information on different habitat preferences, which can be used for appropriate conservation planning.

2.1.2. Skull morphology and morphometrics

Subspecies are usually distinguished by phenotypic traits, and these may include skull morphometry. Guenon skulls are traditionally considered undifferentiated and homogenous (Cardini and Elton, 2007, 2008), and poor species discriminators within a species-rich genus like *Cercopithecus* (Verheyen, 1962; Wood and Richmond, 2000). As a result they have been the subject of few morphological studies. One of the few studies of guenon craniodental morphology was conducted by Verheyen (1962), who analysed 221 adult specimens of 13 guenon species using linear measurements of cranial, mandibular and dental characters. Apart from finding it difficult to identify diagnostic characters and demonstrating marked sexual dimorphism, Verheyen showed that guenon skulls are strongly influenced by allometry – i.e. skulls of similar sizes show parallel shape changes.

Examining guenon skull morphologies can assist in determining whether hard tissue features of the skull map onto the species boundaries that are indicated by calls, ecology, geographic range, and soft tissue. Generally, morphometrics is a powerful tool for identifying and quantifying small inter- and intra-specific differences in primate morphology (e.g.

Cardini *et al.*, 2007). For instance, traditional morphometric studies have identified highly divergent taxa, including *Allenopithecus nigroviridis* (Verheyen, 1962; Martin and MacLarnon, 1988) and *Miopithecus* (Verheyen, 1962), found in molecular analyses to be the most basal divergences of the guenon clade (Tosi *et al*., 2005; Cardini and Elton, 2007).

2.2 Materials and Methods

2.2.1. Data taken from skins

For all specimens represented by skins, I made photographic records of the pelage colouration and recorded any standard body measurements that had been taken by the collectors. These included head-body length, tail length, hind foot length (excluding the claw) and ear length for 15 males and 22 females of *C. cf. albogularis* (see Appendix 1). I also collected body measurements from 8 live males and 12 live females of *C. a. labiatus* (Hogsback village) with the help of Dr Adrian Tordiffe, and used a set of means provided by Butynski *et al*. (2013) for *C. a. erythrachus*, based on specimens from various localities. The museum measurements and those from Hogsback allowed a comparison between *C. a. labiatus* (small form) and *C. a. albogularis* (large form) and were analysed using ANOVA to include both the comparisons between the two forms and potential sexual dimorphism. The analysis was based on absolute measurements to account for differences in body sizes and set up diagnostic characters. However, I also used bivariate regressions against head-body length (size) and ANOVAs and *t*-tests performed on residuals to demonstrate differences in body shape revealed by allometric relationships with body length. This method was only used for tail length, which was the only character to show a significant difference between *C. a. albogularis* and *C. a. labiatus*. Because only mean values were available for *C. a. labiatus*, I compared the two South African subspecies using the means found in Butynski *et al*. (2013) to 95% confidence intervals I calculated for *C. a. labiatus*.

2.2.2. Cranial morphometric methods

Although the South African specimens were the focus of the study, other specimens from this superspecific group were also included to broaden the comparison (see Appendix 2 for specimen details). The evidence obtained from skull morphology is not always conclusive but can distinguish morphotypes which are likely to have had some degree of independent history (Masters and Lubinsky, 1988).

Morphometrics is the quantitative description, analysis, and interpretation of shape and shape variation in biology (Rohlf, 1990), and is an integral component in evolutionary, ecological and developmental studies. Morphometrics is used to reveal patterns of shape variation within and among groups (e.g. species, populations, etc.), and patterns of growth and evolution (e.g. allometry). Scientists use morphometrics to develop and test hypotheses regarding the origin of patterns of variation, quantifying skull morphological variation to understand (1) the functions of various traits with respect to the ecological role of the species examined, and (2) the relationship within and among groups (Rohlf, 1990). Hence, I applied morphometrics to identify potential, subtle morphological differences between the South African samango subspecies.

The first comprehensive book on multivariate morphometrics was published by Blackith and Reyment (1971). The classical studies of this period involved linear measurements. Recently, geometric morphometrics, based on the co-ordinates of landmarks, has become popular. The latter, however, requires expensive equipment, and creates large databases for each specimen. My study, which includes 65 specimens, was performed using linear measurements; my results will serve as the baseline for future geometric studies.

2.2.3. Craniodental data acquisition

Systematists typically wish to measure those aspects of shape that contain information about group membership, and for most organisms, we do not know which those are before the analysis is done. I investigated previous morphometric studies of primates (Masters and Lubinsky, 1988; Masters and Bragg, 2000; Ackermann and Cheverud, 2000, 2002) to identify characters that had proved useful to other researchers, as well as Roberts' (1951) classic list of traditional measurements taken from mammal skulls.

I used Mitotoyo Digimatic digital callipers to record 16 measurements, where possible, from 153 specimens housed in the major museum collections of southern Africa, i.e. Amathole Museum in King William's Town in the Eastern Cape, Durban Natural Science Museum in KwaZulu Natal, Ditsong Museum in the city of Tshwane, Gauteng Province and Natural History Museum of Zimbabwe in Bulawayo, Zimbabwe. In addition to animals from South Africa, I included specimens of other subspecies that were represented (albeit in low numbers) in these collections, so that I could obtain a more comprehensive understanding of morphometric variation in southern samangos. The final sample consisted of 21 *C. albogularis* (not classified to subspecies), 12 *C. a. labiatus*, 9 *C. a. erythrarchus*, 9 *C. a. moloneyi*, 4 *C. a. nyasae*, 9 *C. a. opisthostictus* and 1 *C. a. francensae* (see Appendix 2 for specimen details). Only adults were included in the analysis, and age determination was based on the eruption of a third molar; no specimen included had badly worn teeth. A total of 65 of the 153 specimens had complete craniodental data sets, and these formed the basis of the multivariate study. Subspecies were identified on the basis of their localities, as well as their pelage features. Measurements were repeated until I felt secure about the reading. Details of the measurements are illustrated on Fig. 2.2 and recorded in Table 2.2.

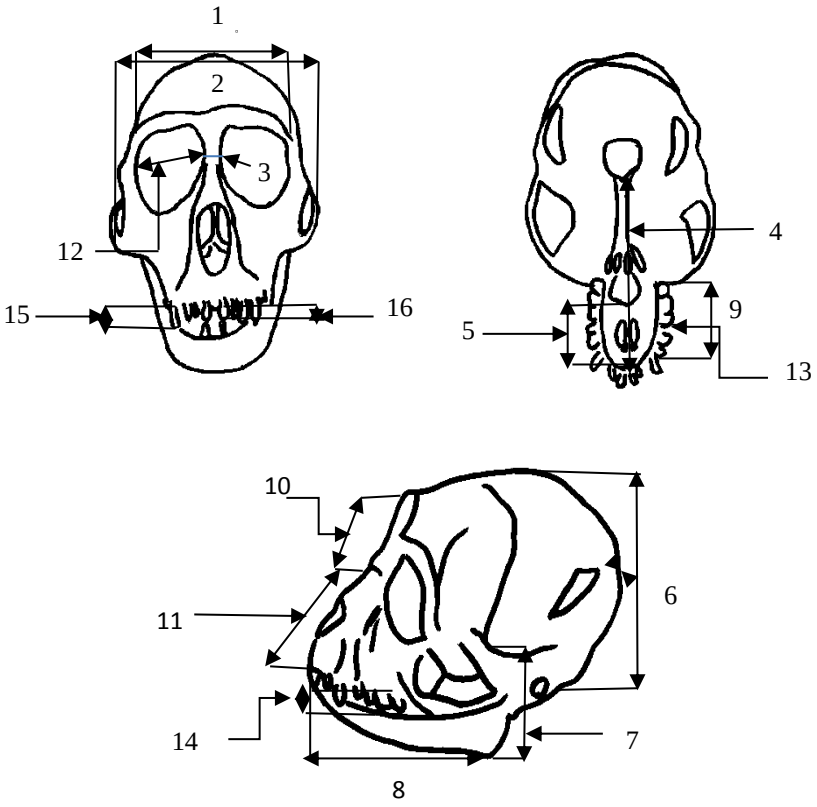

Fig 2.2. Diagram of samango monkeys skull showing measurements recorded for morphometric analysis.

Table 2.1. Craniodental measurements recorded from museum specimens

1. Postorbital constriction: minimum width of braincase, dorsally
2. Zygomatic width: maximum width across zygomatic arches at right angles to long axis of skull
3. Interorbital constriction: minimum width between orbits
4. Basicranial length: from anterior edge of foramen magnum to anterior margin of first incisors
5. Palate length: from back of palate to anterior margin of first incisors
6. Cranial height: maximum height from basioccipital-basishenoid fissure
7. Height of ramus: from apex of coronoid process to lower border of mandible immediately below
8. Length of mandible: from back of angular process to anterior margin of mandibular symphysis
9. Upper cheek tooth series length: from first premolar to third molar
10. Frontal length: from parietal fissure to nasal fissure
11. Nasal length: from frontal fissure to anterior nasal margin
12. Orbital width: maximum width from point of interorbital constriction
13. Molar 1 width: width of occlusal surface of the first upper molar
14. Molar 1 height: from the margin to lower edge of the first upper molar
15. Canine length: maximum length from labial margin
16. Medial incisor length: from labial margin to its edge

2.2.4. Morphometric data analysis

I used a graphic method (histograms) to evaluate the normality of all characters. Moreover, all measurements were linearized by a log-transformation. Discriminant Function Analysis (DFA) can provide a graphical representation of the degree of separation between or among taxa. Because some of characters showed a bimodal distribution, I chose to perform two DFAs (DFA1 and DFA2): DFA1 excluded the bimodal characters, and DFA2 excluded all specimens of the frequency mode that did not include the South African specimens. In DFA, groups for differentiation need to be pre-determined by the researcher. DFA1 was based on the two categories corresponding to the modes of the distributions of the excluded characters; and DFA2 was based on three categories: (a) specimens labelled as *C. a. erythrarchus*, (b) those labelled as *C. a. labiatus*, and (c) all other subspecies of the southern group, all found in the same western part of southern Africa. The latter included some specimens labelled as "*C. m. moloneyi*" and all specimens labelled as "*C. m. nyasae*", all from localities of the west of the distribution of the southern form.

2.2.5. Distribution of museum specimens

The localities of the museum specimens included in my study were mapped using ArcGIS version 10.1 are represented in Fig 2.3 below.

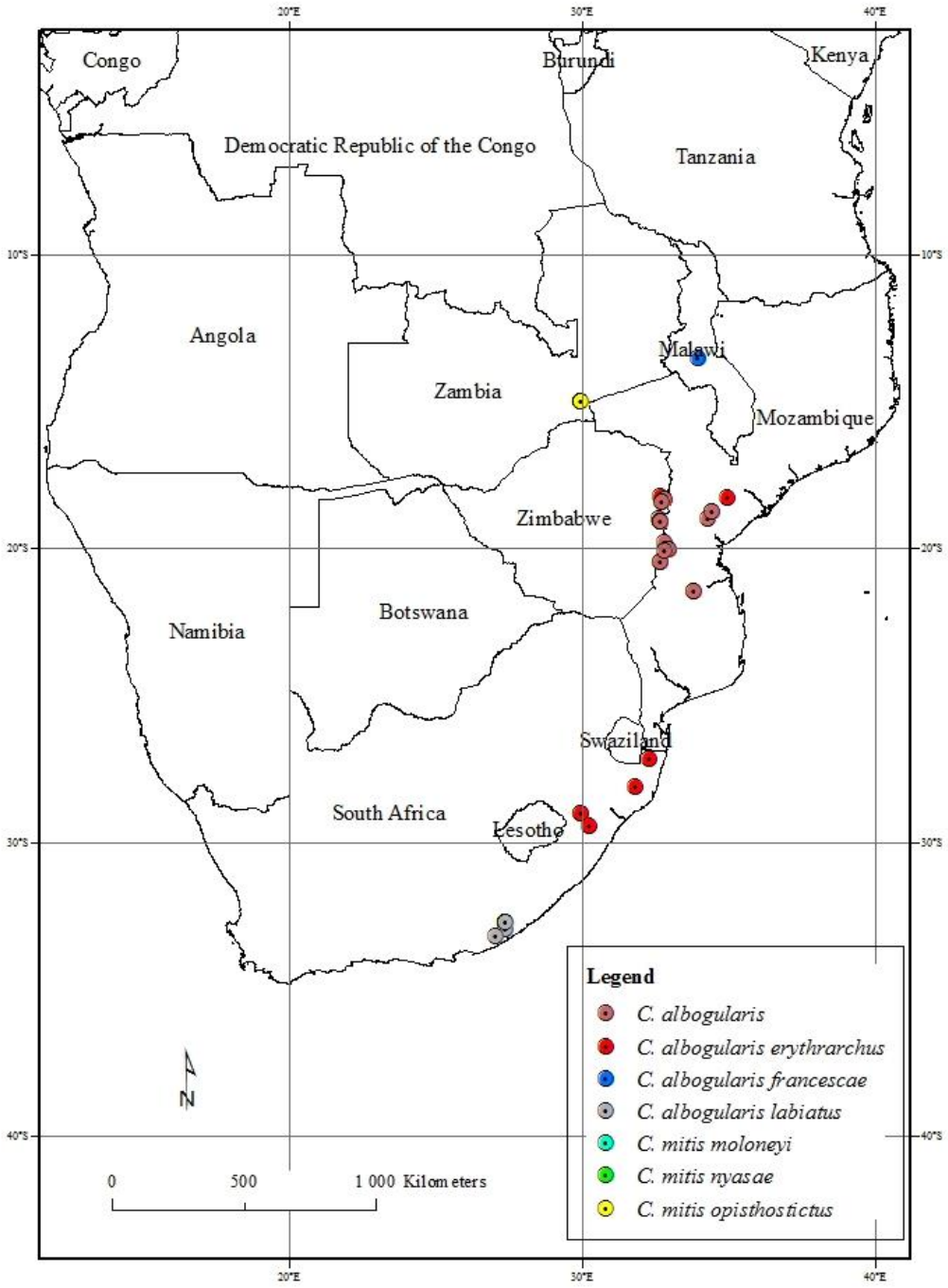

Fig.2.3. Localities of specimens housed in southern African museums

My sample of *C. a. labiatus*, which is distributed from the Eastern Cape to the KwaZulu-Natal midlands and southern Mpumalanga, is restricted to the Eastern Cape only, limiting the amount of morphological variation investigated in my study; but this is due to a paucity of specimens in local museum collections. The range of *C.a. erythrarchus* extends from Limpopo Province and northern KZN into Mozambique and north-eastern Zimbabwe; my sample includes specimens from across this range. *C. a. labiatus* is confined to higher altitude forests and coastal forest of the Afromontane region up to approximately 33˚S (Lawes, 1990), while *C. a. erythrarchus* occupies coastal lowland and riparian forests, as well as low quality swamp forest and mixed woodland on Bazaruto Island (Downs and Wirminghaus, 1997).

2.3. Results

2.3.1. Data taken from skins

The two South African subspecies, *C. a. erythrarchus* and *C. a. labiatus*, are readily distinguishable in terms of pelage characteristics, with *C. a. erythrarchus* resembling the *C. a. albogularis* populations distributed further north into Tanzania; i.e. on the dorsum and the rump, both the Tanzanian and South African east coast specimens have a distinctive russet colour, which is the origin of the name "erythrarchus" (Fig. 2.4).

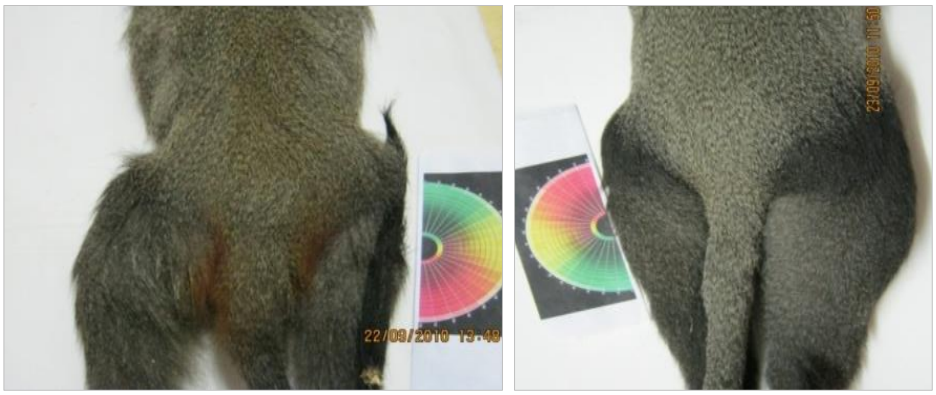

Fig. 2.4. Rump region of *C. a. erythrarchus* (left) and *C. a. labiatus* (right)

2.3.2. Frequency distributions of cranial characters

I found four characters that separated my sample into two distinct groups: Symphysis Length (SL), Interorbital Constriction (IOC), Medial Incisor Length (MIL) and Canine Length (CL). SL showed the most distinct bimodal frequency distribution of all the characters (Fig. 2.5).

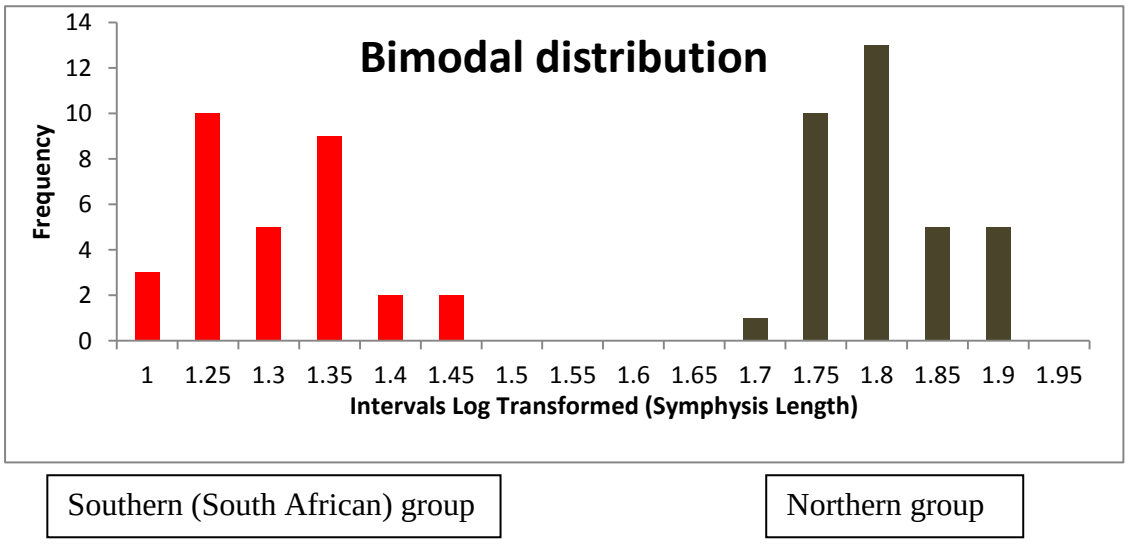

Fig. 2.5. Bimodal frequency distribution of Symphysis Length (SL).

The bimodal frequency distributions separated the sample into two distinct groups that were geographically defined. Along with *C. a. labiatus* and *C. a. erythrarchus*, the southern group, which were smaller in body size, included some *C. mitis moloneyi* and all *C. m. nyasea*, while the northern, larger sized group consisted of *C. m. opisthostictus*, some *C. m. moloneyi* and *C. m. francescae*, and some animals labelled broadly as "*C. mitis*", although some are most likely to be *C. albogularis*.

The fact that symphysis length (SL) was the most distinctive character separating the two geographic groups is deserving of comment. The southern populations had shorter symphyses, while the symphyses of the northern group were consistently longer. In Fig. 2.5 the separation interval is shown as log transformed values 1.50 to 1.65, which is equivalent to

31.6 to 44.7 mm. Animals with SL< 31.6 mm are from the southern group; those with SL > 44.7 mm are from the southern group.

The symphysis length is related to the body size of the species, but may also indicate different masticatory forces operating on the lower jaw, and hence be related to dietary differences. The different habitat tolerances of the taxa suggest that they could have different feeding adaptations.

C. m. moloneyi individuals were included in both groups, which may mean geographic overlap between the smaller southern and larger northern forms. Napier (1981) thought that this subspecies might be transitional between them. Alternatively, it could be a simple matter of misidentification: animals in the study labelled as *C. m. moloneyi* could in fact belong to two distinct species.

My sample also included one specimen of *C. m. francescae* which according to Napier (1981) is one of the southern forms. *C. m. francescae*, a dark grey group with red ear tufts (Ansell, 1960), was synonymized with *C. m. moloneyi* by Schwarz (1928). The fact that my specimen groups with the northern forms suggests that it may have been misidentified.

2.3.3. Multivariate statistical analyses

My data sample was separated into two distinct groups by DFA1. The four diagnostic characters with bimodal frequency distributions (SL, CL, IOC and MIL) (Fig. 2.5), were excluded from this analysis, which was therefore based on 12 characters; nevertheless, the remaining characters which had not shown bimodal distributions also separated the data sample into two distinct groups (Fig. 2.6). I interpret this to mean that my sample consisted of two species within the taxon *C. albogularis*.

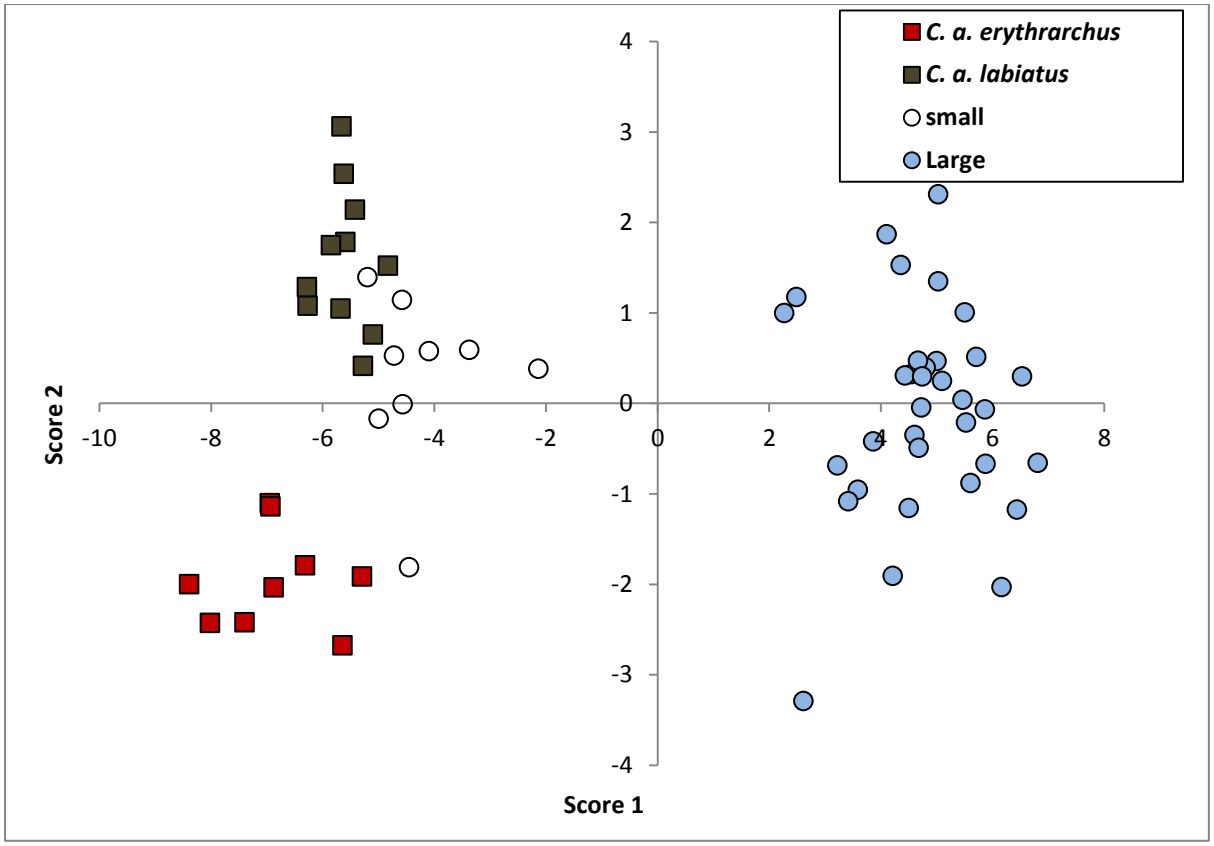

Fig. 2.6. Results of the Discriminant Function Analysis (DFA1) showing two clear groups I interpret as distinct species.

The open circles in the graph represent *C. m. moloneyi* and *C. m. nyasae*, and are designated as "small" in the key. The blue circles, designated "large" in the key, represent the northern species. The two species were separated by score 1 (see Appendix 2), while score 2 separated *C. a. erythrarchus* from *C. a. labiatus.* Most *C. m. moloneyi* and *C. m. nyasae* specimens clustered with *C. a. labiatus*, while one *C. m. moloneyi* specimen (-4.452, -1.813) lies closer to *C. a. erythrarchus*, and is identified as a misclassification in Appendix 3. This analysis hence had a correct classification frequency of 98.5%.

For DFA2 the northern species was eliminated from the analysis, and I explored the possibility of morphometrically defined groups at the infra-specific level of the southern species (consisting of *C. a. labiatus*, *C. a. erythrarchus*, *C. m. moloneyi* and *C. m. nyasae*).

DFA2 separated *C. a. erythrarchus* and *C. a. labiatus* from *C. m. moloneyi*/*C. m. nyasae* on the basis of score 1, and score 2 separated *C. a. labiatus* from *C. a. erythrarchus* (see Fig. 2.7 and Appendix 3). The separation of *C. a. labiatus* from *C. a. erythrarchus* is evidence that their skulls have subtle size and shape differences which may reflect different diets (e.g. different masticatory stresses; *cf.* Lawes *et al.*, 1990; Lawes, 1991) or slightly different developmental rates and conditions. In Fig. 2.7, *C. a. erythrarchus* and *C. a. labiatus* are represented by red and brown squares, respectively, while *C. m. moloneyi* and *C. m. nyasae* are lumped as "other", and represented by open circles. The close morphometric similarity of these specimens shows that the other subspecies is either *C. m. moloneyi* or *C. m. nyasae*. The latter two taxa appear to be sympatric, and in my opinion should be synonymized as *C. m. moloneyi*. *C. m. nyasae* has only been recorded in Malawi (2 specimens), whereas *C. m. moloneyi* has a wider distribution.

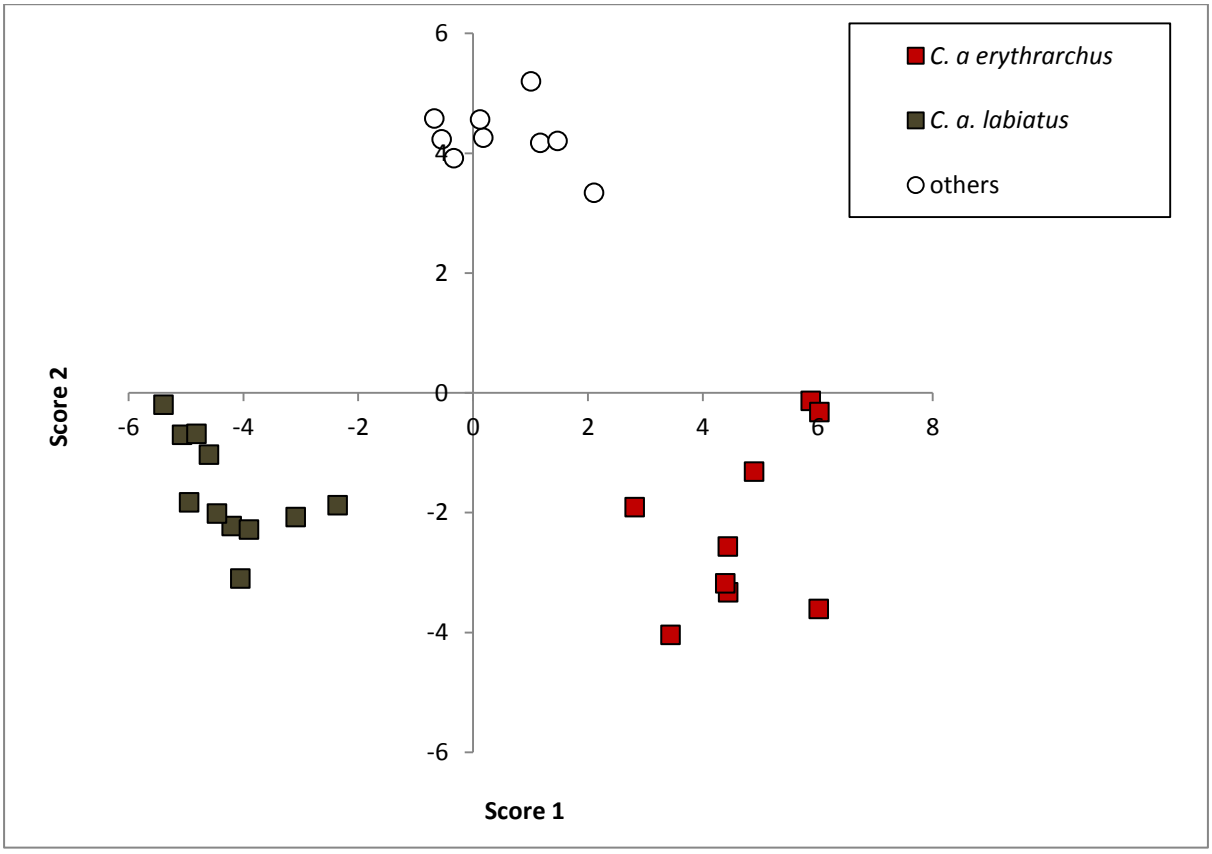

Fig. 2.7. Results of the DFA2 analysis including the southern subspecies

2.3.4. Post-cranial measurements

Because many morphological measurements are correlated with body size, I used a bivariate regression against head-body length to analyse the allometric relationships of my characters using total sample sizes, which varied across characters. The plot revealed an expected positive allometry for body mass, and negative allometries for hind foot length, tail length, and ear length (Fig. 2.8). However, because there was no difference in head-body length between the two forms showing differences in cranial measurements (southern and northern forms), I first used absolute measurements for comparisons when no difference in body size was observed, and size-independent residuals when specimens differed in size.

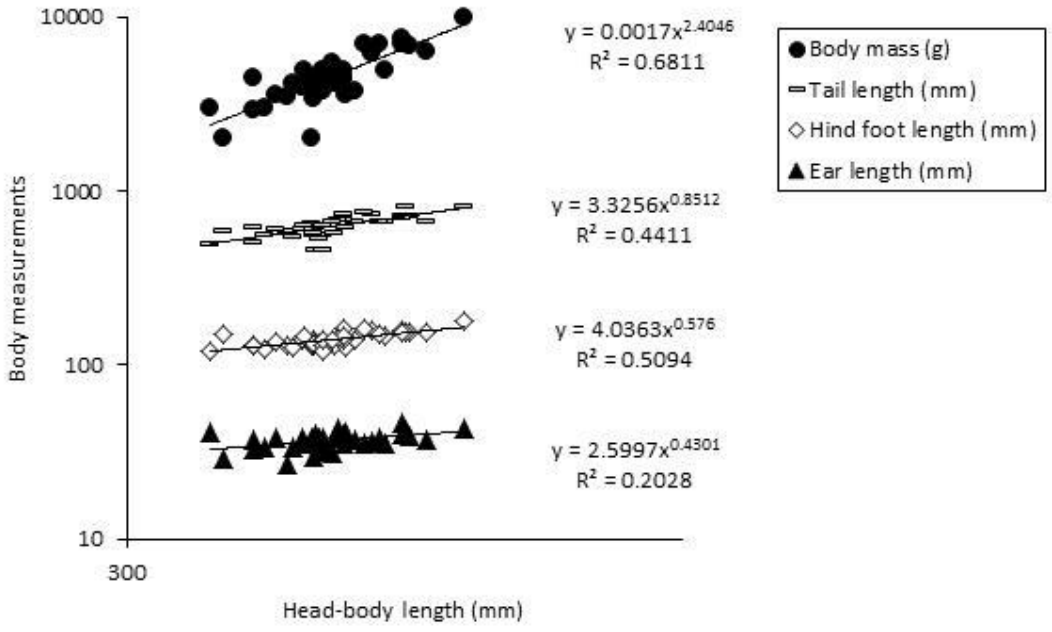

Fig. 2.8. Allometries of the standard body measurements (logarithmic scale) for *C. a. labiatus* (small form) and *C. a. albogularis* (large form)

Measurements taken from museum specimens and my own Hogsback measurements allowed a comparison between *C. a. labiatus* (small form) and *C. a. albogularis* (large form). The results of this comparison using ANOVA to include both the comparisons between the two forms and sexual dimorphism are given in Table 2.2 and show essentially a shorter tail in *C. a. labiatus* without any difference in body size.

Table 2.2. Comparison between absolute body measurements of *C. a. labiatus* (southern form: Hogsback) and *C. a. albogularis* (northern form) including sexual dimorphism

Statistics	Head-body length (mm)	Tail (mm)	Hind foot length (mm)	Ear length (mm)
Model	F4, 49 = 6.6, P < 0.001	F4, 50 = 9.3, P < 0.001	F4, 48 = 9.6, P < 0.001	F4, 49 = 1.4, P = 0.200
Form	F1, 49 = 0.05, P = 0.800	**F1, 50 = 14.20, P < 0.001**	F1, 48 = 1.50, P = 0.200	-
Sex	F2, 49 = 12.0, P < 0.001	**F2, 50 = 10.7, P < 0.001**	F2, 48 = 15.6, P < 0.001	-
form x sex*	F1, 49 = 1.6, P = 0.20	**F1, 50 = 2.9, P = 0.09**	F1, 48 = 3.1, P = 0.08	-

*Interaction between the two factors; bold: significant differences between the southern and the northern forms of *Cercopithecus albogularis*

I compared these data with those recorded in Butynski *et al.* (2013) for *C. a. erythrarchus*. The latter included means calculated from specimens measured throughout the distribution of this subspecies. The results of these comparisons are given in Table 2.3, and show *C. a. erythrarchus* to be the largest form with a significantly longer tail than *C. a. labiatus*.

Moreover, sexual dimorphism in tail length is directly explained by size; sexual dimorphism and tail length were isometrically corrrelated with head-body (HB) length ($N = 58$, $r = 0.687$, $P < 0.001$). The HB-based regression allows a comparison in tail length independently of body size. *C. a. labiatus* shows significantly lower HB-independent residuals of tail length, indicating a difference in body proportion ($t\ 56 = 3.8$, $P < 0.001$). Despite the fact that *C. a. labiatus* has the smallest body size, it is not significantly lighter than *C. a. albogularis* (see Table 2.3).

Table 2.3. Comparisons of body measurements of the three southern *Cercopithecus albogularis*: *C. a. labiatus* and *C. a.nyasae** museum measurements and *C. a. erythrarchus* from Butynski's data (2013)

Form	Sex	Statistics	BM[1] (kg)	HB length (mm)	Tail (mm)	HF legth (mm)	Ear length (mm)	Locality	*N*
C. a. labiatus			4.0	443	550	129	35	Hogsback	12
		95% CI	3.7-4.4	424-461	517-583	126-132	33-38	.	
C. a. erythrarchus	**Females**		3.8	**492**	**671**	**141**	37	Throughout	22
"C. a. albogularis"			4.0	467	657	140	36	Zimbabwe	22
		95% CI	3.3-4.8	451-484	624-689	135-144	34-38	.	
C. a. labiatus			5.2	499	645	147	38	Hogsback	8
		95% CI	3.8-7.2	448-550	574-716	137-157	35-42	.	
C. a. erythrarchus	**Males**		6.9	**555**	**726**	154	39	Throughout	12
"C. a. albogularis"			6.3	523	726	153	39	Zimbabwe	15
		95% CI	3.6-11.0	483-563	666-786	144-163	35-42	.	

[1] Reduced sample size: 5 males and 14 females for *C. a. albogularis*; 95% CI = confidence interval; and bold = statistically significant

*"*C. a. albogularis*" from Zimbabwe is probably *C. a. nyasae*. BM = Body mass, HB = Head-body, HF = Hind foot.

2.4. Discussion and conclusion

My analysis has shown clear morphological differences between *C. a. labiatus* and *C. a. erythrarchus*. The two subspecies differ in pelage colouration, but also in cranial shape, body sizes and proportions. In particular, *C. a. labiatus* can be described as slightly smaller, short-tailed and stocky compared to the long-tailed slender northern forms, which may be an adaptation to cold environment such as montane forest.

The southern group appears to be made up of three morphometrically distinct groups, *C. a. erythrarchus* (in Mozambique, northern KwaZulu-Natal), *C. a. labiatus* (Eastern Cape, KwaZulu-Natal midlands and southern Mpumalanga) and *C. m. moloneyi* (possibly Limpopo, north-eastern Zimbabwe to the northern shore of Lake Malawi, and Zambia) (Butynski *et al.*, 2013). The northern *C. albogularis* subspecies are distinguishable from the southern taxa with a high degree of reliability (98.5%), suggesting that *C. albogularis* consists of more than one species. Since the *C. albogularis* type specimen was described from Zanzibar island (Sykes, 1831), the southern "species" – if my hypothesis is valid – will need a new species nomen. Since *C. a. labiatus* was described by Isadore Geoffroy-St. Hilaire in 1843, while *C. a. erythrarchus* was described by Wilhelm Peters in 1852 and *C. a. moloneyi* by Philip Sclater in 1893, I believe the new species should be named *C. labiatus*, with subspecies *C. l. erythrarchus*, C. *l. labiatus* and *C. l. moloneyi.* This proposal deserves more extensive investigation, particularly at the genetic level. In the following chapter I describe my preliminary genetic studies of this problem.

Chapter 3: Genetic Differentiation

3.1. Introduction

3.1.1. Genetic distance/divergence vs. *reproductive isolation*

Systematists attempt to describe the variation within taxa as well as the historical relationships among them. Different approaches to species definitions have yielded quite divergent assessments of the extant biodiversity (Peterson and Navarro-Siquenza, 1999). Genetic distance, or the degree of dissimilarity between the genetic compositions of taxa, therefore appears to some to be an ideal systematic tool (Ayala, 1975). Genetic distance measures only the degree of genetic divergence between taxa and is not explicitly bound to any of the current species concepts or process-laden theories (Ferguson, 2002).

Sufficient genetic distance is supposed by many to indicate reproductive isolation between presumptive species (reproductive isolation is taken as the combination of pre-mating and post-mating isolation in the sense of Mayr, 1963). Reproductive isolation is held to occur because of the slowly accumulating number of genetic differences between lineages (Wu and Hollocher, 1998). Reproductive isolation is the keystone of the Biological Species Concept (BSC) (Masters, 1993). Accordingly, BSC species are separated by isolating mechanisms that prevent interbreeding. Isolating mechanisms can be grouped into pre-mating isolating mechanisms (e.g. behavioural and physical incompatibility) and post-mating isolating mechanisms (e.g. genetic incompatibility resulting in death of embryos).

Some authors (Sasa *et al.,* 1998) have published evidence of a correlation between genetic divergence and reproductive isolation between species. That there should be a relationship between these two conditions is a reasonable assumption, since genomic incompatibilities will result from the accumulation of genetic differences at many loci over a

long period of time (Ferguson, 2002). However, random genetic drift (e.g. as a result of habitat fragmentation) does not necessarily result in reproductive isolation. Wu and Hollocher (1998) have argued that large numbers of genes, spread over most or all of the chromosomes, are responsible for reproductive isolation between close taxa. Speciation appears to involve a re-organization at the genome level.

According to the Recognition Concept (RC) of species, characters involved in specific-mate recognition will remain stabilized in time and space. This implies that there will be no predictable relationship between the amount of genetic divergence and the species identity of the individuals. However, genetic divergence between populations could imply different adaptive and survival capacities, which in turn would have important implications for their conservation. If the taxa *C. a. labiatus* and *C. a. erythrachus* are not just subspecific variants (i.e. adapted to slightly different habitat conditions) but distinct species, then their gene pools will be separate and their adaptive and survival capacities are different.

3.1.2. Conservation genetics

Conservation genetics is a combination of genetic concepts and tools which are used to solve problems in conservation biology. I used mtDNA sequences, which represent one of the most commonly used tools in conservation genetics. Other methods include nuclear genes, microsatellites and, recently, single-nucleotide polymorphisms (SNPs). Genetic sequences are the most common source of data for phylogenetic reconstructions, and the identification of monophyletic groups (Oyler-McCance and Leberg, 2005). A summary of the uses of genetics in conservation biology is presented in Table 3.1.

Table 3.1. Different applications of genetics in conservation biology (modified from Desalle and Amato, 2004).

Role in Conservation Biology	**Sub-disciplines**
Minimising inbreeding and loss of genetic variation	Population genetics
Identifying populations of concern	Population genetics
Resolving population structure	Population genetics
Resolving taxonomic uncertainty	**Systematics**
Defining management units within species	**Systematics**
Detecting hybridization (genetic pollution)	Population genetics/ systematics
Detecting and defining invasive species	Population genetics/ systematics
Defining sites and genotypes for re-introduction	Population genetics or systematics
Use in conservation forensics	Systematics
Estimation of population size and sex ratio	Population genetics
Establishing parentage, pedigree analysis	Population genetics
Understanding population connectivity	Population genetics/ systematics
Use in the management of captive populations	Population genetics
Understanding relationships of focal groups of taxa	Systematics
Implementing genotoxicity studies	Population genetics
Increasing the reproductive capacity of organisms	Population genetics

My study has focused on systematic issues in the South African *C. albogularis* populations, and falls under "resolving taxonomic uncertainty" and "defining management units within species".

Identifying genetic variation within and among populations is important for the prevention of inbreeding in endangered species like samango monkeys. It also makes it possible to identify the presence and effect of genetic drift, and whether a population has undergone selection or a recent genetic bottleneck (Hedrick and Miller, 1992; Hedrick 1999; Oyler-McCance and Leberg, 2005). For hundreds of years taxonomic classifications have been done on the basis of morphological and behavioural characteristics, but classifications relying solely on morphological characterization can be misleading (Avise, 1989). Masters and colleagues (Masters, 1993; Masters and Lubinsky, 1988) have argued that morphology is generally a bad species indicator in nocturnal primates that use non-visual signals to communicate and identify one another. Cercopithecoid monkeys are diurnal, and morphology is a crucial part of their signalling system. Nevertheless, combining genetic data with morphological and behavioural information is the best way of insuring a reliable identification of species identity (Oyler-McCance and Leberg, 2005).

3.1.3. Conservation Units

Ryder (1986) invented the term Evolutionary Significant Unit (ESU) with the goal of conserving adaptive genetic variance within species, through conserving unique populations below the species level, to ensure that historical and geographical variants are recognized and protected (Avise, 1989; Waples,1995, 1998; Fraser and Bernatchez, 2001). The evolutionary significant unit (ESU) is prominent in the conservation of natural animal populations. Some authors advise against the translocation of individuals between ESUs (Avise, 1994; Ryman, 1991), in case it disrupts local adaptations. However, not translocating individuals exposes small populations to the risk of inbreeding depression (Edmands, 2007).

Another conservation unit of interest is the management unit (MU); i.e. populations of conspecifics with relatively low levels of connectivity, so that each population should be

monitored and managed on its own (Taylor and Dizon, 1999; Palsbøll *et al.*, 2006). The most commonly used MU definition is that of Moritz (1994, p. 373): "populations with significant divergence of allele frequencies at nuclear or mitochondrial loci, regardless of the phylogenetic distinctiveness of the alleles"; i.e. the lineages are demographically independent.

3.2 Materials and Methods

3.2.1. Sampling localities

Tissue samples (ear punches) were taken from samango monkeys resident in two localities in Hogsback, Eastern Cape, South Africa (32° 35' 0" South, 26° 57' 0" East; see Fig. 3.1). The main locality was in the village, near the old Woodlands grocery store, and the animals were designated as the village troop (V in Table 3.2). The second capture site was near Hobbiton where the animals encounter elements of natural forest, and designated as the forest troop (F in Table 3.2). The subscripts in Table 3.2 show the sequence in which the animals were trapped at each site. The majority of samples came from the village troop, since it was easier to trap animals that were habituated to humans and used to eating food like oranges, which were used as bait. Furthermore, it was easier to place the traps under trees in the village than in the dense undergrowth of the forest.

Fig. 3.1. Map showing Hogsback and the two capture localities (red dots): Village troop (left) and Forest troop (right); the "forest" at Swallowtail Falls was somewhat degraded.

3.2.2. Sampling method

The monkeys were sampled using a "trap and release" method, using steel cage traps designed for live monkey capture (Fig. 3.2).The traps were activated when the animal touched the bait (an orange), which was placed on a trigger. Captured monkeys were anaesthetized by a licensed veterinarian (Dr Adrian Tordiffe of the National Zoological Gardens of South Africa), using Zolatil 100 or Ketamine. An ear notch of approximately 2x2 mm was taken from each individual and stored in eppendorf tubes containing 95% ethanol. All samples were stored at 4˚C until DNA extraction. After sampling, the monkeys were placed in a safe area where they were protected from cold, predators and conspecific rivals. The monkeys gradually recovered from the anaesthetic within a period of approximately 1 hour.

The trapping, sedation and sampling methods were approved by the Animal Ethics Committee at the University of Fort Hare, and carried out under a permit issued by the relevant provincial conservation authority, Eastern Cape Parks.

Fig 3.2. Cage trap with an orange as bait in Hogsback

3.2.3. Genetic protocols

3.2.3.1. DNA extraction. This protocol involves sample lysis, DNA binding and purification. I used a Roche High Pure PCR Template Preparation Kit (Roche Diagnostics), and followed the manufacturer's instructions as follows: the elution buffer was warmed to ≥ 70˚C before extraction and kept at a constant temperature throughout the process. A pair of tweezers was used to place the samples on a petri dish, which were blotted dry with a paper towel. Each sample was put into a nuclease-free 1.5 ml micro-centrifuge tube, containing 200 µl tissue lysis buffer and 40 µl reconstituted Proteinase K. The mixture was vortexed immediately for ± 20 seconds and incubated for 1 hour at 55 ˚C until each sample was digested completely. After digestion, 200 µl binding buffer was added. The mixture was then vortexed again for ± 20 seconds and incubated for 10 minutes at ≥ 70 ˚C. After incubation, 100 µl isopropanol was added using a micro-pipette and the tube contents were mixed. The liquid was then then added to the upper reservoir of a High Filter assembly and centrifuged for 1min at 8 000 x g.

After centrifugation, the filter tube was placed in a new collection tube and the flow-through liquid was discarded with the used collection tube; then 50 µl inhibitor removal buffer was added to the upper reservoir of the filter tube and centrifuged for 1 min at 8, 000 x g. The filter tube was again placed in a new collection tube, and 500 µl wash buffer was added to the upper reservoir of the filter tube and centrifuged for 1 min at 8 000 x g. This step was repeated. After discarding the flow-through liquid the second time, the filter tube was placed in the same collection tube and centrifuged for an additional 10 s at full speed (16,000 x g). This was to ensure the removal of all residual wash buffer. To elute the DNA, the filter tube was placed in a clean 1.5-ml micro-centrifuge tube and 200 µl pre-warmed elution buffer was added to the upper reservoir of the filter tube. The assembly was then centrifuged for 1 min at 8 000 x g. DNA quantification was done with a Nanodrop® ND-1000 Spectrophotometer v3.7 to evaluate the success of the extraction procedures, in terms of DNA concentration and absorbance (level of purity). A sufficient DNA concentration was considered to be ≥ 25 ng/µl with an absorbance ≥ 1.8 (see Table 3.2).

3.2.3.2. Gel electrophoresis. A Schott bottle was used to mix agarose gel and TAE (Tris Acetate EDTA), and was placed in a micro-wave oven to dissolve the agarose completely. The bottle was then cooled under running tap water, and the solution was poured into a gel container. Air bubbles were removed before the gel could set, and a comb was placed across the container. The comb was removed after the gel had set, and the gel was placed in 1 x TAE running buffer; 6 µl of each DNA sample were mixed with 1 µl of bromophenol blue to stain the DNA. The samples were loaded into the comb wells of the agarose gel, and the gel was run at 80 volts for 20 minutes. DNA samples migrate toward the positive pole, since they are negatively charged because of their phosphate backbones. Visualisation was done under a UV light.

Table 3.2. DNA concentration and absorbance of the extracted samples. Samples used in further analyses are denoted by *.

Sample ID	**Concentration (ng/µl)**	**Absorbance: A260/A280**
*F_1	13.6	1.67
*F_2	8.2	1.39
*F_3	9.5	1.56
*F_4	12.0	1.47
F_5	8.7	1.36
F_6	11.1	1.42
*F_7	22.5	1.49
*F_8	20.1	1.55
V_1	29.3	1.43
V_2	32.3	1.53
V_3	23.0	1.40
*V_4	16.6	1.45
V_5	9.7	1.68
*V_6	38.9	1.60
V_7	18.2	1.36
V_8	21.8	1.42
V_9	17.3	1.40
V_{10}	25.7	1.50
*V_{15}	12.1	1.55
V_{17}	8.3	1.91
*V_{22}	50.7	1.8

The eluted DNA was used immediately to run polymerase chain reaction (PCR).

3.2.3.3. Gene sequencing. Following DNA extraction and quantification, a section of the mitochondrial 12S rRNA gene was amplified. The primers L 1091(Light Chain) and H 1478 (Heavy Chain) (Kocher *et al.*, 1989) were ordered from Inqaba Biotec, and were composed of the following sequences:

L 1091- 5′-AAAAAGCTTCAAACTGGGTTAGATACCCCACTAT-3′

H 1478- 5′-TGACTGCAGAGGGTGACGGGGGGTGTGT-3′

3.2.3.4. Polymerase Chain Reaction (PCR). PCR is a biochemical technology that is used to amplify DNA to produce many copies of a particular sequence. The method relies on thermal cycling, i.e., cycles of repeated heating and cooling, which drives rapid DNA replication. It involves a sample (the DNA template); two primers, i.e. short DNA fragments that have sequences complimentary to 3' ends of each of the sense and anti-sense strand of the DNA template; Taq polymerase (an enzyme isolated from the bacterium *Thermus aquaticus* which is heat resistant up to 70˚C) to drive the replication; deoxynucleoside triphosphates (dNTPs, i.e. nucleotides with triphosphate groups to make new DNA strand); buffer solution, which creates suitable chemical environment for optimum activity and stability of DNA polymerase. The DNA generated provides more template for replication, and is exponentially amplified. The reaction I used is summarized in Table 3.3.

Table 3.3. Standardized reaction setup recommended for KAPA2G Robust HotStart ReadyMix PCR reactions.

Component	**Final concentration**	**Volume in a 25 µl reaction**
PCR grade water	-	Up to 25.0 µl
2X KAPA2G Robust HotStart ReadyMix (Contains 2mM $MgCl_2$ at 1X)	1X	12.5 µl
Forward primer (10 µM)	0.5 µM	1.25 µl
Reverse primer (10 µM)	0.5 µM	1.25 µl
Template DNA	As needed	4 µl

The amounts displayed in Table 3.3 were adjusted depending on the number of samples to be analysed per session, to form a master mix. For instance, in the case of 12 samples, each value was multiplied by 12. Template DNA was added to make a total volume of 25 µl. The minimum/ maximum melting temperature (Tm) for both primers was 53˚C. The cycle parameters were: 3 min at 95˚C for initial denaturation, amplification reactions for 35 cycles of 15 s at 95˚C, 15 s at 53˚C and 15 s at 72˚C, with a final elongation step of 10 minutes at 72˚C, and with a 4˚C hold (short-term fridge storage). The thermocycle I used is summarized in Table 3.4.

Table 3.4. The thermal cycling protocol used in this study.

Step	Temperature (˚C)	Time	Number of cycles
Initial denaturation	95	3 min	1
Denaturation	95	15 sec	35
Primer annealing	53	30 sec	
Extension	72	15 sec	
Final extension	72	10 min	1
Cooling	4	Infinite	1

The amplified products were viewed with a known size-standard ladder on a 1% agarose gel. PCR products of adequate quality were purified using the BioSpin PCR Purification Kit (BioFlux, Tokyo, Japan) to remove any unused primers, dNTPs and denatured DNA. The purified products were then labelled using the ABI PRISM® Big Dye® Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems Division, Perkin-Elmer, and Foster, CA, USA). The labelling reaction mixture consisted of 2.5 µl Big Dye Terminator v3.1 sequencing buffer, 0.5 µl Big Dye® Terminator mix, 1 µl of 2 mM primer, 4 µl dH_2O and 2 µl DNA template. The labelled products were purified using the ZR DNA Sequencing Clean-up™ Kit (Zymo Research, Orange, CA, USA) and sequenced on an ABI 3130 Genetic Analyzer.

3.2.3.5. PCR purification. The PCR or enzymatic reaction product was transferred to 1.5 ml micro-centrifuge tubes, and binding buffer was added to the PCR product in a ratio of 2:1 and vortexed. (The maximal volume added should not exceed 200 µl; mine was 10 µl). The mixture was applied to the spin column by decanting or pipetting and centrifuged for 1 minute at 6,000 x g. The flow-through liquid was discarded. The spin column was then

washed with 650 µl wash buffer and centrifuged for 1 min at 12,000 x g. Once again, the flow-through liquid was discarded. This step was repeated once, and centrifuged for an additional 1 min at 12,000 x g. The spin column was then transferred to a sterile 1.5 ml micro-centrifuge tube. This step removed any residual liquid in the column. Subsequently, 50 µl elution buffer was added and the mixture left to stand for 1 min at room temperature. After it had been centrifuged for a further 1 min at 12,000 x g, the sample was ready for sequencing reactions.

3.2.3.6. Clean-up of sequencing reactions. 240 µl of sequencing binding buffer was added to a 5 – 20 µl sequencing reaction. The mixture was transferred to a zymo-spin™ IB column in a collection tube and centrifuged at 13, 000 rpm for 1 min. A quantity of 300 µl sequencing wash buffer was added to the column, and centrifuged again at 13, 000 rpm for 1 min. After 10 µl HiDi had been added directly to the column matrix, the column was transferred to a 1.5 ml micro-centrifuge tube and centrifuged at 13, 000 rpm for 1 min to elute the DNA. The ultra-pure DNA was then loaded into the sequencer.

3.2.3.7. Sequence alignment. Raw gene sequences were viewed and assembled using the software *Geneious Pro 5.4* (Drummond *et al.*, 2011). This programme is an integrated, cross-platform bioinformatics software package. The assembled sequences were aligned using alignment explorer M5 (nucleotide alignment *fasta* programme) of the *Geneious* software. The sequences were trimmed to 274 bp to ensure uniform sequence lengths during analysis.

3.2.4. Phylogenetic analysis

Cercopithecus albogularis, C. mitis and C. nictitans have been showed to be closely affiliated (Tosi *et al.*, 2005), and are sometimes grouped under the *C. mitis* superspecies (Sineo, 1990). In my phylogenetic reconstruction, I compared the sequences of the 10 samangos captured in Hogsback with three other 12S sequences retrieved from GenBank: two individuals identified

as *C. mitis* (accession numbers AF164878 and L35197), and one *C. nictitans* (L35199). The latter three animals were not provenanced, and their species identity cannot be verified with certainty. However, it appears likely that they came from the Central African Republic (van der Kuyl *et al.*, 1995, 2000), which would support their GenBank identification. The outgroup consisted of two vervet monkey samples collected by Riël Coetzer and Prof. Paul Grobler of the Department of Genetics at the University of the Free State (Coetzer, 2010). These samples were designated T_2 and T_3.

A Maximum Likelihood analysis was performed in Mega v.5 (Tamura *et al.*, 2011), to identify the relationships between the individual samples and sample sites. The Mega software was also used to find the most appropriate substitute model, which in this case was the model of Tamura and Nei (1993). Node reliability was tested using the boostrap method, with 1,000 bootstrap replications.

3.3. Results

Of the 21 tissue samples collected, only 10 contributed to the analysis. Those that did not yield sufficient quantities of DNA (see Table 3.2) or did not show satisfactory electrophoresis results, were eliminated. Others were discarded during sequence alignment, primarily because they were too short.

The Maximum Likelihood analysis yielded the tree represented in Fig. 3.3. The 10 Hogsback individuals, both from the Forest and Village troops, formed a strongly supported clade (98% bootstrap support). This clade was reconstructed as the sister to the *C. mitis* clade, but with no support (44%), meaning that this node is essentially unresolved. The two *C. mitis* individuals formed a strongly supported clade (99%), testifying to the presence of two

distinct genetically-defined groups. *C. nictitans* forms the basal divergence of the *C. mitis* supergroup, but once again without support. The two vervets cluster with 100% support, and are highly divergent from the samangos.

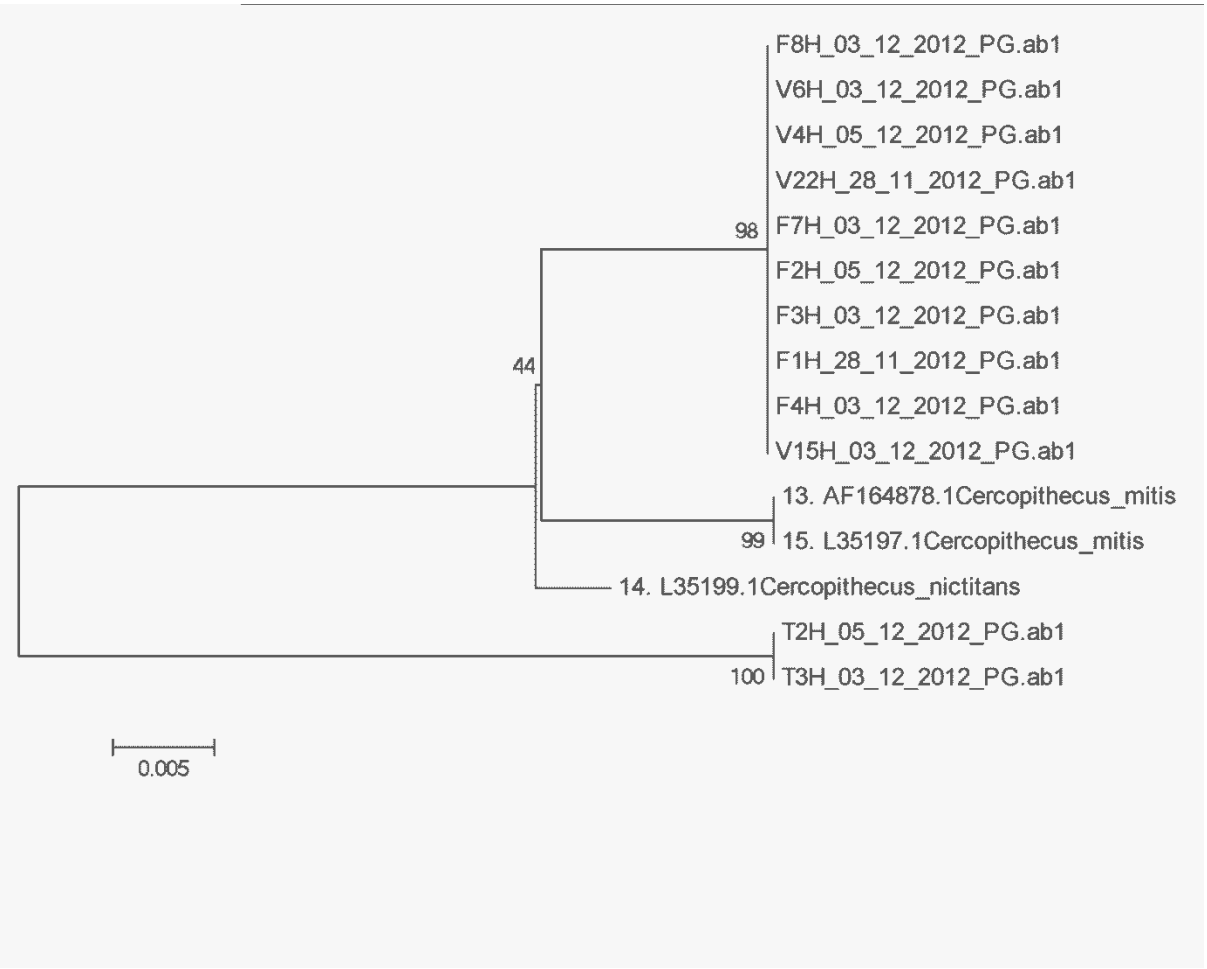

Fig. 3.3. Phylogenetic tree recovered from the Maximum Likelihood analysis

3.4. Discussion and conclusions

The mt 12S rRNA gene sequences has been used successfully to resolve phylogenetic questions in a range of organisms from invertebrates to primates (e.g. Ballard *et al.*, 1992; van der Kuyl *et al.*, 1995; DelPero *et al.*, 2000), chiefly because the primers appear to be almost universal. However, it was not an ideal gene for this study. The ribosomal secondary

structure contains both paired and unpaired regions (DelPero *et al.*, 2000), and the paired regions are extremely stable, rarely showing substitutions, while the unpaired regions have a much higher substitution rate. Depending on the section of the gene that is sequenced, the sequences may show very little variation (as in this case), or much higher rates of variation. My study did not detect variation among the 10 animals sampled in Hogsback; it was able to cluster the different taxa, but it could not resolve the relationships between the different taxa, with the exception of the outgroup. The lack of resolution of the deeper nodes could also be a result of the fact that our sequence was very short (274 bp). Ideally, I would have studied a less conserved section of the mtDNA molecule, specifically the D-loop, which changes very rapidly. However, the sequence structures of *C. albogularis* are so different from those of *Chlorocebus* that the primers of the latter failed to identify the D-loop sequence of the *C. albogularis* samples, and the PCR procedure failed in consequence. Given a more extensive time period, I could try to design suitable primers, but I believe this is a task beyond the scope of my dissertation.

Earlier taxonomists recommended synonymizing *C. mitis* with *C. albogularis* (Napier, 1981), because there are cases of hybridization between *C. mitis stuhlmanni* and *C. albogularis kolbi* across the rift valley (Kingdon, 1971) and because Booth (1968) considered the population around the Lake Manyara as being hybrids between *C. m. stuhlmanni* and *C. a. kinobotensis*. However, karyological studies by Dutrillaux *et al.* (1980, 1982, 1998) and by Sineo (1990) identified consistent karyotypic differences between the two species. Although Sineo (1990) found both taxa to have a diploid number of 72, their karyotypes differ by a pair of pericentric inversions. These are chromosomal rearrangements that include the centromere, and may cause alignment problems during meiosis; hence they may affect interfertility. My genetic data suggest that they are not, in fact, exchanging genes, and are distinct species.

Despite the lack of bootstrap support at deeper nodes on my phylogenetic tree, its topography concurs with Sineo's (1990) phylogenetic assessments based on karyology. Sineo held that *C. albogularis* is more closely related to *C. mitis* than to *C. nictitans*.

My results also concur with the guenon taxonomy compiled in February 2000 during the IUCN/SCC Primate Specialist Group's workshop, *Primate Taxonomy for the New Millennium*, held in Orlando, Florida (Grubb *et al*., 2003), in which five species, *C. nictitans*, *C. mitis*, *C. dogetti*, *C. kandti*, and *C. albogularis* were listed as members of this *Cercopithecus mitis* supergroup.

More genetic work is clearly needed to resolve the phylogenetic relationships within the *C. mitis* supergroup. However, my preliminary genetic data indicate that the southern samangos are distinct from the *C. mitis* of West Africa, while my morphometric study suggests they may also be distinct from *C. albogularis* in East Africa. Molecular and karyological studies comparing the genomes of Hogsback "*C. a. labiatus*" with the neighbouring "*C. a. erythrarchus*" and Zanzibar *C. albogularis* would be extremely enlightening.

Chapter 4: Habitat and Distribution of Samango Monkeys

4.1. Guenon distributions

Guenons are widely distributed in Africa, ranging from Senegal in the west to Ethiopia in the east, and southwards, including islands like Zanzibar, down to the province of the Eastern Cape in South Africa. *C. albogularis* appears to have reached Ethiopia, Somalia and South Africa during a relatively wet period, when there was a continuous belt of rain forest linking these areas. Today *C. albogularis* shows a disjunct distribution due to increasing dryness which has fragmented the forest into isolated tracts, several of which provide habitats for recognized subspecies (Rahm, 1970). The cercopithecine species group with the widest distribution is *Chlorocebus aethiops*, since it can tolerate dry forests. Some subspecies of *C. albogularis* can survive in dry forests, and this species group is second in terms of range size (Booth, 1968).

Phylogenetic studies have grouped *Cercopithecus nictitans* (the putty-nosed monkey), *C. mitis* (the blue monkey) and C. *albogularis* as a species group (van der Kuyl *et al.*, 1995, 2000; Tosi *et al.*, 2005; Butynski *et al.*, 2013) that is widely distributed across a range of forested habitats in sub-Saharan Africa (Fig 4.1). None of the proposed species or subspecies appear to have overlapping distributions. Animals distributed to the east of the Rift Valley from south-eastern Somalia to the eastern part of South Africa are part of the *C. albogularis* complex, whereas those to the west, including the taxa labelled ”stuhlmanni” and “opisthostictus”, are classified as subspecies of *C. mitis*. According to my study, the “*C. albogularis*” of East Africa and South Africa/Mozambique are unlikely to represent a single species, and the smaller-bodied southern group should be assigned the name *C. labiatus*, including the populations currently classified as *C. a. erythrarchus* and *C. a. labiatus*.

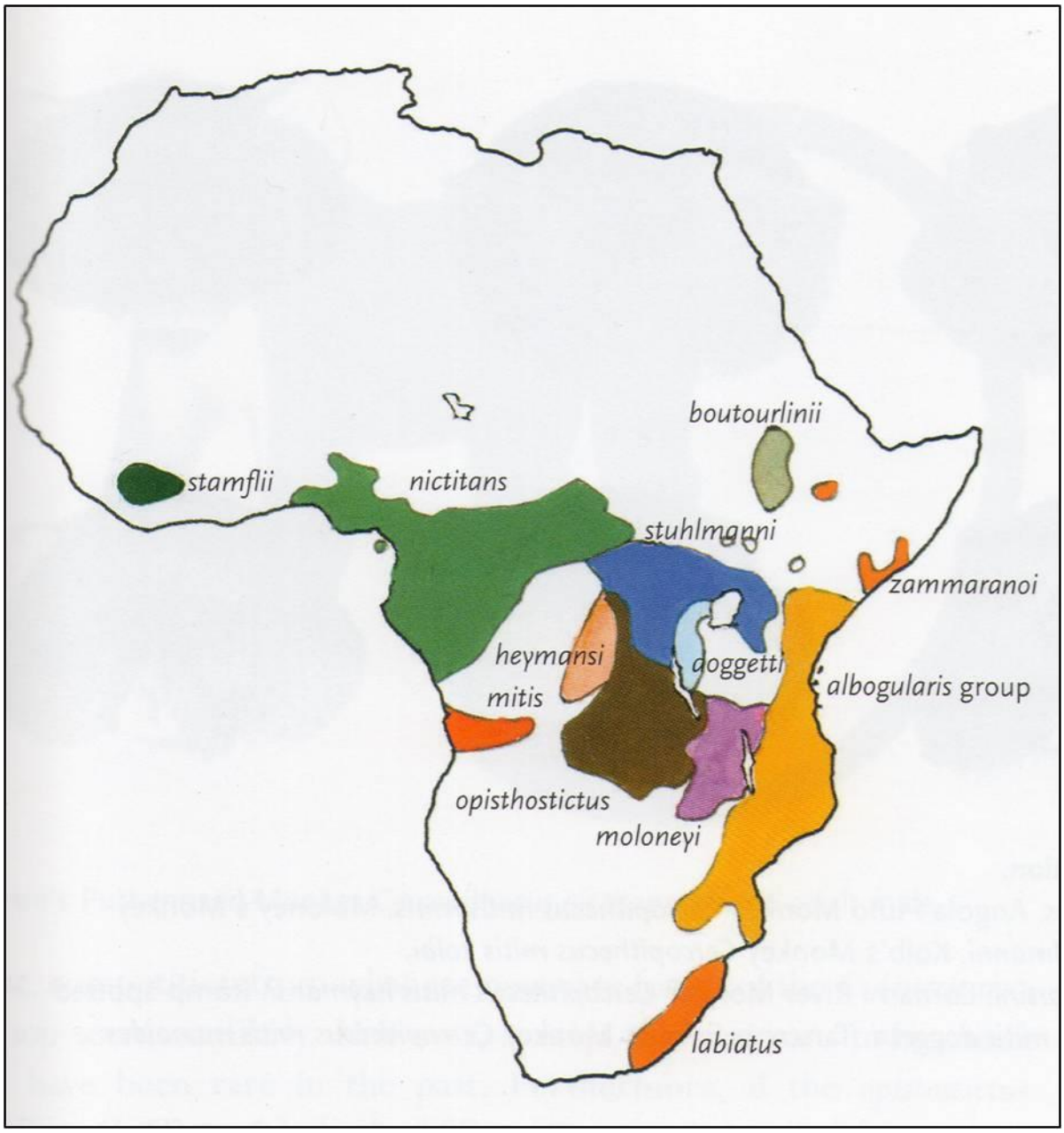

Fig 4.1. Distribution of the *Cercopithecus nictitans/mitis/albogularis* species group, from Butynski *et al.* (2013)

4.3. Discussion

4.3.1. Forest history of southern Africa and the distribution of samango monkeys

The distributions of forest mammals have been influenced by the vegetation changes that accompanied climatic changes during the Pleistocene, from 1.8 Mya (Stuckenberg, 1969).

Samango monkeys have restricted and even anomalous distribution patterns when compared with the extent of Pleistocene coastal and inland forests. For instance, no samango monkeys are found in the Knysna and Tsitsikama forests of the Western Cape. *C. a. erythrarchus* is chiefly found in lowland forests, but also occurs in the Afromontane forests of the Limpopo province and the eastern highlands of Zimbabwe, which theoretically should be more suited to *C. a. labiatus*.

Lawes (1992) proposed two possible emigration routes for samangos into the South African region, that are directly related to the timing and the spread of Afromontane and Indian Ocean coastal forest, respectively, in the past. Glacial maxima fragmented evergreen forest, creating refuge areas for forest animals and plants (Lawes, 1992). Glacial and interglacial cycles lasted about 100 000 years (Tyson, 1986), so approximately 20 such cycles would have occurred within the past 2 Myr.

Of the two forest types that characterize the South African subspecies, Afromontane forest is more ancient and more persistent than the Indian Ocean coastal forest belt, which was established after the last glacial maximum (Tinley, 1985). Afromontane forest was established before the last glacial maximum (Moreau, 1963; White, 1981). Thus, *C. a. labiatus* and *C. a. erythrarchus* may have different radiation patterns. Lawes (1992) presents evidence that palaeoclimatic events have affected both forest and forest mammal distributions over the past 150,000 years, especially the ranges of samangos in southern Africa.

4.3.2. Radiation of C. a. labiatus *and* C. a. erythrarchus

Lawes (1992) proposed that *C. a. labiatus* radiated into the southern African subregion during the hypothermal period of Afromontane forest and woodland expansion, prior to the last glacial maximum. The southern limit of subspecies' range is the Pirie forest in the Eastern Cape. The fact that the taxon is not present in the Knysna and Tsitsikama forests

suggests that there was a climatological and/or vegetation barrier (e.g. grassland, semi-arid karoo bushveld) that prevented the animals' spread to the southernmost extent of the coastal forest. Lawes (1992) hypothesized further that *C. a. erythrarchus* radiated from east African coastal *C. albogularis* populations relatively recently, as far as southern Mozambique coastal plain and into Maputo, since coastal lowland forest began to establish itself on the Mozambique plain only after 8000-6000 years BP. The Eastern Highlands of Zimbabwe are not inhabited by *C. a. labiatus* because the Afromontane migration corridor is interrupted both north and south by the Zambezi and Limpopo river valleys, respectively, where the Mozambican coastal plain cuts in sharply toward the eastern highlands. *C. a. labiatus* in the Zimbabwean highlands before the last glacial period were probably eliminated by the cold temperatures, and later replaced by coastal tropical *C. a. erythrarchus* (Lawes, 1992). During this process, *C. a. labiatus* and *C. a. erythrarchus* must have encountered each other in the Limpopo province (Roberts, 1951), which may represent a zone of co-existence between the two taxa. If they do co-occur, this would be evidence of distinct species, since subspecies, by definition, cannot be sympatric. Furthermore, the radiation of *C. a. erythrarchus* south of Maputo along the coast into KwaZulu-Natal appears to have been halted by the St. Lucia and Umfolozi river systems. The latter give some evidence that the radiation of *C. a. erythrarchus* into the southern African subregion occurred after the last glacial maximum. Any further southward movement of *C. a. erythrarchus* was prevented by a lack of vegetation corridors or presence of *C. a. labiatus* in western forests, e.g. Hluhluwe. *C. a. labiatus* is intimately linked to the extent and distribution of Afromontane forest, but not entirely restricted to them. The lack of evidence for secondary contact (e.g. a chromatic cline) between the subspecies in northern KZN implies that Afromontane and Indian Ocean coastal belt forest in the region have been separated throughout the history of the South African subspecies.

4.4. Summary and conclusions

The historical biogeography of the South African samango monkeys appears to have been characterized by four major events:

- Separation of *C. albogularis* between glacial hypothermals from the equatorially located mother population, and the radiation and differentiation of the group deep into southern Africa with *C. a. labiatus* reaching the Eastern Cape;
- The fragmentation of the Afromontane forests during the last glacial maximum (18,000 years BP) with *C. a. labiatus* populations being restricted to refugia in the Eastern Cape, possibly coastal scarp forests of KZN, and south-eastern Mpumalanga;
- The initial populations of *C. a. labiatus* in the eastern highlands of Zimbabwe and possibly Limpopo, were eliminated by harsh dry conditions during the last glacial maximum 18,000-12,000 years BP;
- The radiation of tropical elements southward along the coastal plain (6000 years BP to present), with *C. a. erythrarchus* reaching as far as Umfolozi swamp region in the south and moving up into the eastern highlands of Zimbabwe and also into the eastern Limpopo province (Lawes, 1992).

The best means available to test this hypothesis, is to use rapidly mutating mtDNA sequences, like the D-loop. Further research into the history of the South African subspecies and the timing of their divergences should be studied using molecular dating techniques. I hope to do this in my future research.

Chapter 5: Discussion and Conclusions

5.1. Introduction

5.1.1. South African primate biodiversity

Biological variation is distributed morphologically, phylogenetically and geographically. In my study I have looked at morphological and genetic variation in samango monkeys (*Cercopithecus albogularis*) in southern Africa. Morphometric and genetic variation were compared spatially, and assessed in relation to habitat differences. *C. a. labiatus* and *C. a. erythrarchus* were distinguished morphologically and ecologically, although we had no samples to test their genetic status. Genetic divergence would imply different adaptive and survival capacities, which in turn would have important implications for their conservation.

Samango monkeys are an important and vulnerable component of South Africa's biodiversity. Despite the attention biodiversity has received in recent years, there has been little, if any, reduction in the degree of threat faced by life on Earth. Although, there is a greater awareness of how acute the threat is and how pervasive are its implications, many South Africans still wish to destroy the country's endemic primates. One of our tasks as primatologists is to expose and challenge those misconceptions.

Biodiversity is usually estimated in terms of species, although there is little agreement among biologists as to how these taxa are defined. Many biologists follow 'isolation concept' of species ('Biological Species Concept'): this concept defines species as ''groups of interbreeding populations that are reproductively isolated from other such groups''. Thus, to diagnose BSC species, researchers look for characters that confer reproductive isolation, which may include any characters that reduce the probability of successful mating ('pre-mating isolating mechanisms' and 'post-mating isolating mechanism'). The term 'isolating

mechanism' invokes selection as the force driving the evolution of such characters for the function of isolation, but many (like hybrid sterility) cannot be selected (Darwin, 1872).

With the advance in molecular technology, more and more systematists base their diagnosis of species on genetic distance measures, but there is no fixed amount of genetic divergence that denotes a speciation event. Speciation has more to do with genomic re-organization than random divergence (Masters, 1998; Ferguson, 2002).

In contrast the Recognition Concept of species described by Paterson (1978, 1985) defines a species as the most inclusive population of biparental organisms that share a common fertilisation system. Adherents of this view, like me, identify species by characters that function to bring about fertilisation. In mobile organisms, like samango monkeys, we should concentrate on characters of the premating 'Specific-Mate Recognition System' (SMRS), which constitute a subset of the characters of the fertilisation system. These are behaviours like social organisation and communication, which I would like to focus on in future studies. While pre-mating and post-mating reproductive isolation exist, these phenomena are never the product of selection for isolation, but are incidental by-products or effects of divergence. The RC predictions are as follows: 1) SMRS characters are species-specific and environment-specific. 2) Speciation occurs when the SMRS is no longer effective in the new environment. 3) Because of the stability of the SMRS, speciation is always accompanied by niche shifts. 4) The new species should not have the same ecological niche as its parent species. Samango monkeys can be used to explore this speciation model because of their extensive and recent radiation and, sometimes, sympatric distributions. However, in this dissertation, I have restricted my scope to investigate indirect evidence that the monkeys have distinct gene pools (i.e. morphology and genetics).

5.2. Taxonomy and samango conservation biology

5.2.1. Samango taxonomy

In order to work meaningfully with biodiversity, it is necessary to place organisms in groups, or taxa, which reflect their evolutionary relationships. Taxonomists frequently disagree about groupings and heated arguments can occur. My results accord in general with the guenon taxonomy compiled in February 2000 during the IUCN/SCC Primate Specialist Group's workshop, *Primate Taxonomy for the New Millennium*, held in Orlando, Florida (Grubb *et al*., 2003), in which five species, *C. nictitans*, *C. mitis*, *C. dogetti*, *C. kandti*, and *C. albogularis* were listed as members of this *Cercopithecus mitis* supergroup. However, my study also suggests that the detailed taxonomy used by most primatologists to characterize the southern African samango monkeys is incorrect. *Contra* Butynski *et al.* (2013), they are unlikely to be subspecies of the west African *Cercopithecus mitis* superspecies, and placing them under this species obscures the endangered status of the Afromontane subspecies. According to karyological studies (Sineo, 1990; pers. comm.), the South African samangos are derived from the East African superspecies group *C. albogularis*, although they probably are a distinct species, and should be referred to *C. labiatus.*

The identification of subspecies is essential for the research and conservation of these taxonomic units at appropriate geographical scales. A proper understanding of species status is a crucial tool in biodiversity studies, and is particularly important in the case of threatened or endangered populations. Subspecies or morphologically similar species (i.e. cryptic or sibling species) may have very different resource requirements and ecological tolerances. If *C. a. erythrarchus* and *C. a. labiatus* are considered as trivial variants of a single homogeneous group, they are regarded as "of least concern" by conservation authorities, but

C.a. labiatus is certainly not "of least concern", and requires a different conservation strategy from its close relative.

5.2.2. Conservation Management Units

In terms of the management units (MUs) identified by conservationists, *C. a. erythrarchus* and *C. a labiatus* appear to be distinct MUs, although we have yet to provide the kind of genetic data that would identify them as demographically independent lineages. Nevertheless, patterns of morphological variation within species can also play an important role in in revealing evolutionary diversification, and morphology is often used in the classification of species (Ackermann & Cheverud, 2002). I have shown that the skulls of *C. a. labiatus* and *C. a. erythrarchus* can be reliably distinguished using 16 linear measurements. The two subspecies are also readily distinguishable in terms of pelage characteristics, with *C. a. erythrarchus* resembling the *C. albogularis* populations distributed further north into Tanzania. Despite this superficial similarity, my multivariate analysis of skull characters clusters the two South African populations to the exclusion of animals from Zimbabwe and Mozambique. Taking all these morphological differences together, *C. a. labiatus* can be described as slightly smaller, shorter-tailed and stocky compared to the long-tailed slender northern forms, as well as *C. a. erythrarchus.* These differences in *C. a. labiatus* may be adaptations to cold environments such as montane forest.

5.2.3. Genetic variation

The mt 12S rRNA gene sequences have been used successfully to resolve many phylogenetic questions, and the primers are almost universal, making it relatively easy to copy the gene sequences using PCR. However, in terms of its inherent conservatism, it was not an ideal gene for this study. My study did not detect variation among the 10 animals sampled from Hogsback, and although it was able to cluster the different taxa, it could not resolve the

relationships among the different species, with the exception of the outgroup. Future research should involve identifying primers for a less conserved section of the mtDNA molecule, specifically the D-loop, which changes very rapidly. Molecular and karyological studies comparing the genomes of Hogsback "*C. a. labiatus*" with the neighbouring "*C. a. erythrarchus*" and Zanzibar *C. albogularis* would be extremely enlightening.

5.3. Distribution and biogeographic history

According to palaeoclimatic studies of the forests of southern Africa (Lawes, 1990, 1992), the two South African subspecies appear to have migrated into the country at very different times. The "Afromontane" *C. a. labiatus* arrived earlier, as the spread of these inland forests occurred during the hypothermal period that preceded the last glacial maximum (18 000 – 12 000 years ago). The Indian Ocean coastal forests, home to *C. a. erythrarchus*, spread very much later – 8000 to 6000 years ago. According to this hypothesis, the phylogeographic divergences within *C. a. labiatus* should thus be deeper than those within *C. erythrarchus*. This is a hypothesis I hope to test in future research.

5.4. Conclusion

Surprisingly little systematic work has been done on South Africa's samango monkeys, despite their importance to the country's ecosystems and their vulnerability. During my research for this dissertation, I have laid the ground work for future studies involving morphological, genetic and biogeographic investigation. I hope to carry the work forward in future.

References

Ackermann, R. R. & Cheverud, J. M. 2000. Phenotypic covariance structure in tamarins (Genus *Saguinus*): a comparison of variation patterns using matrix correlation and common principal component analysis. *American Journal of Physical Anthropology* **111**: 489–501.

Ackermann, R. R. & Cheverud, J. M. 2002. Discerning evolutionary processes in patterns of tamarin (Genus *Saguinus*) craniofacial variation. *American Journal of Physical Anthropology* **117**: 260 – 271.

Agapow, P. M., Bininda-Emonds, O. R. P., Crandall, K. A., Gittleman, J. L., Mace, G. M., Marshall, J. C. & Purvis, A. 2004. The impact of species concepts on biodiversity studies. *The Quarterly Review of Biology* **79**: 161-179.

Ansell, W. F. H. 1960. Contributions to the mammalogy of Northen Rhodesia. *Occasional Papers of the National Museums of Southern Rhodesia* (B) **24**: 351-398.

Avise, J. C. 1989. A role for molecular geneticists in the recognition and conservation of endangered species. *Trends in Ecology and Evolution* **4**: 279-289.

Avise, J. C. 1994. *Molecular Markers, Natural History and Evolution*. Chapman and Hall, New York.

Ayala, F. J. 1975. Genetic differentiation during the speciation process. *Evolutionary Biology* **8**: 1-78.

Ballard, J. W. O**.,** Olsen, G. J., Faith, D. P., Odgers, W. A., Rowell, D. M. & Atkinson, P. W. 1992. Evidence from 12*S* ribosomal RNA that onychophorans are modified arthropods. *Science* **258**: 1345-1348.

Bearder, S. K., Honess, P. E. & Ambrose, L.1995. Species diversity among galagos with special reference to mate recognition. In: *Creatures of the Dark: The Nocturnal Prosimians*, ed. L. Alterman, G. A. Doyle and M.K. Izard, pp. 331-352. Plenum Press, New York.

Benton, M. J. 2007. The Phylocode: Beating a dead horse? *Acta Palaentologica Polonica* **52**: 651-655.

Blackith, R. E. & Reyment, R. 1971. *Multivariate Morphometrics*. Academic Press, London.

Booth, C. P. 1968. Taxonomic studies of *Cercopithecus mitis* Wolf (East Africa). *National Geographic Society Research Reports* **37**: 51.

Butynski, T. M., Kingdon, J. & Kalina, J. (eds) 2013. *Mammals of Africa. Volume II: Primates.* Bloomsbury Publishing, London.

Cardini, A. & Elton, S. 2007. Sample size and sampling error in geometric morphometric studies of size and shape. *Zoomorphology*. **126**: 121-134.

Cardini, A. & Elton, S. 2008. Does the skull carry a phylogenetic signal? Evolution and modularity in the guenons. *Biological Journal of the Linnean Society* **93**: 813–834.

Cardini, A., Jansson, A.U. & Elton, S. 2007. A geometric morphometric approach to the study of ecogeographical and clinal variation in vervet monkeys. *Journal of Biogeography* **34**: 1663–1678.

Cheverud, J. M. 1995. Morphological integration in the saddle back tamarin (*Saguinus fuscicollis*) cranium. *American Naturalist* **145**: 63-89.

Cheverud, J. M. 1996. Developmental integration and the evolution of pleiotropy. *American Zoologist* **36**: 44-50.

Coetzer, W. G. 2012. *Patterns of Genetic Diversity in Vervet Monkeys* (Chlorocebus aethiops) *from the South-eastern Regions of South Africa.* MSc dissertation, University of the Free State, Bloemfontein, South Africa.

Darwin, C.R. 1872. *On the Origin of Species by Natural Selection, or the Preservation of Races in the Struggle for Life*, 6th edn. John Murray, London.

DelPero, M., Masters, J. C., Cervella, P., Crovella, S., Ardito, G. & Rumpler Y. 2000. Phylogenetic relationships among the Malagasy lemuriforms (Primates: Strepsirrhini) as indicated by mitochondrial sequence data from the 12S rRNA gene. *Zoological Journal of the Linnean Society* **133**: 83-103.

DeSalle, R. & Amato, G. 2004. The expansion of conservation genetics. *Nature Reviews of Genetics* **5**: 702-712.

Downs, C. T. & Wirminghaus, J. O. 1997. The terrestrial vertebrates of the Bazaruto Archipelago, Mozambique: a biogeographical perspective. *Journal of Biogeography* **24**: 591-602.

Drummond, A. J., Ashton, B., Buxton, S., Cheung, M., Cooper, A., Duran, C., Field, M., Heled, J., Kearse, M., Markowitz, S., Moir, R., Stones-Havas, S., Sturrock, S., Thierer, T. & Wilson, A. 2011. *Genious v 5.4*, [Available online at http:www.genious.com, downloaded 15 November 2012].

Dutrillax, B., Couturier, J. & Chauvier, G. 1980. Chromosomal evolution of 19 species or sub-species of Cercopithecinae. *Annales de Genetique* **23**: 133-143.

Dutrillaux, B., Couturier, J., Muleris, M., Lombard, M. & Chauvier, G. 1982. Chromosomal phylogeny of forty-two species or sub-species of cercopithecoids (Primates, Catarrhini). *Annales de Genetique* **25**: 96-109.

Dutrillaux, B., Muleris, M. & Couturier, J. 1998. Chromosal evolution of Cercopithecinae. In: *A Primate Radiation: Evolutionary Biology of African Guenons*, ed. A. Gautier-Hion, J. P. Bourlière & J. Kingdon, pp. 150-159. Cambridge University Press, Cambridge.

Edmands, S. 2007. Between a rock and a hard place: evaluating the relative risks of inbreeding and outbreeding for conservation and management. *Molecular Ecology* **16**: 463-475.

Ferguson, J. W. H. 2002. On the use of genetic divergence for identifying species. *Biological Journal of the Linnean Society* **75**: 509 – 516.

Ferrari, S. F. 1993. Ecological differentiation in the Callitrichidae. In: *Marmosets and tamarins: systematics, ecology and behaviour*, ed. A. B. Rylands, pp. 314-328. Oxford University Press, Oxford.

Fraser, D. J. & Bernatchez, L. 2001. Adaptive evolutionary conservation: towards a unified concept of defining conservation units. *Molecular Ecology* **10**: 2741- 2752.

Friedman, Y. & Daly, B. 2006. *Red Data Book of the Mammals of South Africa: A Conservation Assessment*. 2nd edn. Endangered Wildlife Trust, South Africa.

Gaston, K. J. & Spicer, J. I. 2004. *Biodiversity: An Introduction*. Blackwell Publishing, Oxford.

Geoffroy Saint-Hilaire, I. 1843. Description de mammifères nouveaux ou imparfaitment connus de la collection du Musėum d' Histoire naturelle, et remarques sur la classification et les caractères des Mammifères. *Archives du Muséum d'Histoire Naturelle, Paris* **2**: 485-592.

Grantham, T. 2007. Is macroevolution more than successive rounds of microevolution? *Palaeontology* **50**: 75–85.

Groombridge, B. 1992. *Global Biodiversity: Status of the Earth's Living Resources*. World Conservation Monitoring Centre, Cambridge, UK.

Groves, C. 2001. *Primate Taxonomy*. Smithsonian Institution Press. Washington, D.C.

Grubb, P., Butynski, T. M., Oates, J. F., Bearder, S. K., Disotell, T. R., Groves, C. P. & Struhsaker, T. T. 2003. Assessment of the diversity of African primates. *International Journal of Primatology* **24**: 1301-1357.

Hansen, T. F. & Martins, E. P. 1996. Translating between microevolutionary process and macroevolutionary patterns: the structure of interspecific data. *Evolution* **50**: 1404-1417.

Hedrick, P.W. & Miller, P.S. 1992. Conservation genetics: techniques and fundamentals. *Ecological Applications* **2**: 30-46.

Hedrick, P.W. 1999. Perspective: highly variable loci and their interpretation in evolution and conservation. *Evolution* **53**: 313-318.

Hershkovitz, P. 1977. *Living New World Monkeys (Platyrrhini): with an introduction to primates, Vol. 1*. University of Chicago Press, Chicago.

Hill, W. C. O. 1966. *Primates. Comparative anatomy and taxonomy. Vol. IV. Catarrhini, Cercopithcoidea, Cercopithecinae*. Edinburgh University Press, Edinburgh.

Irwin, D. E., Irwin, J. H. & Price, T. D. 2001. Ring species as bridges between microevolution and speciation. *Genetica* **112-113**: 223-243

Kingdon, J. 1971. *East African Mammals: an Atlas of Evolution in Africa, Vol. 1. Primates.* Academic Press, London.

Kingdon, J. 1997. *The Kingdon Field Guide to African Mammals*. Academic Press, San Diego.

Kingdon, J., Gippoliti, S., Butynski, T. M., Lawes, M. J., Eeley, H., Lehn, C. & De Jong, Y. 2008. *Cercopithecus mitis.* In: IUCN 2009. *IUCN Red List of Threatened Species.* Version 2009.1.

Kocher, T. D., Thomas, W. K., A., Edwards, S. V., Pääbo, S., Villablanca F.X. and Wilson, A.C. 1989. Dynamics of mitochondrial DNA evolution in animals: amplification and sequencing with conserved primers. *Proceedings of the National Academy of Sciences, USA* **86**: 6196-6200.

Lande R. 1979. Quantitative genetic analysis of multivariate evolution applied to brain: body size allometry. Evolution **33**: 402-416.

Lawes, M. J., Henzi, S. P. & Perrin, M. R. 1990. Diet and feeding behaviour of samango monkeys (*Cercopithecus mitis labiatus*) in Ngoye forest, R.S.A. *Folia Primatologica* **54**: 57-69.

Lawes, M.J. 1990. The distribution of the samango monkey (*Cercopithecus mitis erythrarchus* Peters, 1852 and *Cercopithecus mitis labiatus* I. Geoffroy, 1843) and forest history in southern Africa. *Journal of Biogeography* **17**: 669-680.

Lawes, M.J. 1991. Diet of samango monkeys (*Cercopithecus mitis erythrarchus*) in the Cape Vidal dune forest, South Africa. *Journal of Zoology* **224**: 149-173.

Lawes, M.J. 1992.The socioecology and conservation of the samango monkey (*Cercopithecus mitis*) in Natal, South Africa. *Biological Conservation* **60**: 197-270.

Martin, R. D. & MacLarnon, A. M. 1988. Comparative quantitative studies of growth and reproduction. *Symposia of the Zoological Society of London* **60**: 39-80.

Masters J. C. 1991. Loud calls of *Galago crassicaudatus* and *G. garnetti* and their relation to habitat structure. *Primates* **32**: 153-167.

Masters, J. C.1993. Primates and paradigms: problems with the identification of genetic species. In: *Species, Species Concepts, and Primate Evolution*, ed. W. H. Kimbel & L. B. Martin, pp. 43-64. Plenum Press, New York.

Masters, J. C. 1998. Speciation in the lesser galagos. *Folia Primatologica* **69** (Suppl.1): 357-370.

Masters, J. 2000. The role of selection in speciation. In: *Evolutionary Genetics from Molecules to Morphology*, ed. R. S. Singh and C. B. Krimbas, pp. 513-531. Cambridge University press, New York.

Masters, J. C. & Bragg, N. P. 2000. Morphological correlates of speciation in bush babies. *International Journal of Primatology* **21**:793-813.

Masters, J. & Lubinsky, D. 1988. Morphological clues to genetic species: multivariate analysis of greater galago sibling species. *American Journal of Physical Anthropology* **75**: 37-52.

Masters, J. C. & Rayner, R. J. 1993. Competition and macroevolution: the ghost of competition yet to come? *Biological Journal of the Linnean Society* **49**: 97-98.

Mayr, E.1963. *Animal Species and Evolution*. Harvard University Press, Cambridge, MA.

McNamara, K. J. & McKinney, M. L. 2005. Heterochrony, disparity, and macroevolution. *Paleobiology* **31**: 17-26.

Meester, J. & Setzer, H. W. 1971. *The Mammals of Africa: An Identification Manual*. Smithsonian Institution Press, Washington, DC.

Meester, J. A. J., Rautenbach, I. L., Dippenaar, N. J. & Baker C. M. 1986. *Classification of the Southern African Mammals*. Transvaal Museum Monograph No. 5. Transvaal Museum, Pretoria.

Moore, A. J. & Cheverud, J. M. 1992. Systematics of the *Saguinus oedipus* group of the bare-face tamarins: Evidence from facial morphology. *American Journal of Physical Anthropology* **89**: 73–84.

Moreau, R. E.1963. Vicissitudes of the African biomes in the Late Pleistocene. *Proceedings of the Zoological Society of London* **141**: 395-421.

Moritz, C. 1994. Defining 'evolutionary significant units' for conservation. *Trends in Ecology and Evolution* **9**: 373-375.

Myers, N., Mittermeier, R. A, Mittermeier, C. G., da Fonseca, G. A. B. & Kent, J. 2000. Biodiversity hotspots for conservation priorities. *Nature* **403**:

Napier, P.H.1981. *Catalogue of Primates in the British Museum (Natural History) and Elsewhere in the British Isles. Part II, Family Cercopithecidae: Subfamily Cercopithecinae*. Trustees of the British Museum (Natural History), London.

Oyler-McCance S, J. & Leberg, P. L. 2005. Conservation genetics in wildlife management. In: *Techniques for Wildlife Investigations and Management, 6th edn,* (ed.) C. E. Braun, pp. 632-657. The Wildlife Society, Bethesda.

Palsbøll, P. J., Bérubé, M and Allendorf, F.W. 2006. Identification of management units using population genetic data. *Trends in Ecology and Evolution* **22**: 11-16.

Paterson, H. E. H.1978. More evidence against speciation by reinforcement. *South African Journal of Science* **74**: 369-371.

Paterson, H. E. H. 1985. The recognition concept of species. In: *Species and Speciation*, ed. E. S. Vrba, pp. 21-29. *Transvaal Museum Monograph* **4**, Transvaal Museum.

Peters, W. C. H. 1852. Zoologie. 1. Säugethiere. *Reise nach Mossambique*. G. Reimer, Berlin.

Peterson, T. A. & Navarro- Siquenza, A. G. 1999. Alternate species concepts as bases for determining priority conservation areas. *Conservation Biology* **13**: 427- 431.

Pocock, R.I. 1907. A monographic revision of the monkeys of the genus *Cercopithecus.* *Proceedings of the Zoological Society of London* 1907: 677-746.

Rahm, U. H. 1970. Ecology, zoogeography, and systematics of some African forest monkeys. In: *Old World Monkeys: Evolution, Systematics and Behaviour*, ed. J. R. Napier and P. H. Napier, pp. 589-628. Academic Press, New York.

Roberts, A. 1951. *The Mammals of South Africa*. Trustees of "The Mammals of South Africa" Book Fund, South Africa.

Rohlf, F. J. 1990. Rotational fit (Procrustes) methods. *Proceedings of the Michigan Morphometrics Workshop* **2**: 227-36. Ann Arbor, MI: University of Michigan Museums.

Ryder, O. A. 1986. Species conservation and systematics: the dilemma of subspecies. *Trends in Ecology and Evolution* **1**: 9-10.

Ryman, N. 1991. Conservation genetics considerations in fishery management. *Journal of Fish Biology* **39** (Suppl. A): 211-224.

Sasa, M. M., Chippindale, P. T. & Johnson, N. A. 1998. Patterns of post-zygotic isolation in frogs. *Evolution* **52**: 1811-1820.

Schwarz, E. 1928. Notes on the classification of the African monkeys of the genus *Cercopithecus* Erxleben. *Annals and Magazine of Natural History* **10**: 649-663.

Schwarz, E. 1933. *Cercopithecus mitis* Wolf für *Simia leucampyx* Fischer. *Zeitschrift für Säugetierkunde* **8**: 279.

Sclater, P. L. 1893. On a new African monkey of the genus *Cercopithecus*, with a list of known species. *Proceedings of the Zoological Society of London* 1893: 243-258.

Sineo, L. 1990. The banded karyotype of *Cercopithecus mitis maesi* compared with the karyotypes of *C. albogularis samango* and *C. nictitans stampflii*. *International Journal of Primatology* **11**: 541-552.

Skinner, J.D. & Chimimba, C.T. 2005. *The Mammals of the Southern African Subregion*. 3rd edn. Cambridge University Press, Cambridge.

Smithers, R. H. N. 1986. *The Mammals of the Southern African Subregion*. University of Pretoria Press, Pretoria. South Africa.

Statistics South Africa. Census 2011. Provinces at a glance. Stats SA Library Cataloguing-in-Publication (CIP) Data. Report no: 03-01-43. 82 pp. [Available online: http://www. statssa.gov.za, Downloaded 13 March 2013].

Stuckenberg, B. R. 1969. Effective temperature as an ecological factor in southern Africa. *Zoologica Africana* **4**; 145-197.

Sundevall, C. J. 1845 *Cercopthecus samango* Wahlberg. *Öfver*s. *Kongliga Vetenskaps-akademien Förh. Stockholm* **1**: 160-161.

Sykes, W. H. 1831. Characters of a new species monkey (*Semnopithecus?*). *Proceedings of Zoological Society London* . pp105- 106.

Tamura K. & Nei M. 1993. Estimation of the number of nucleotide substitutions in the control region of mitochondrial DNA in humans and chimpanzees. *Molecular Biology and Evolution*. (10): 512-526.

Tamura K., Peterson D., Peterson N., Stecher G., Nei M., and Kumar S. 2011. MEGA5: Molecular Evolutionary Genetics Analysis using maximum likehood, evolutionary distance, and maximum parsimony methods. Molecular Biology and Evolution, 28 (10): 2731-2739.

Taylor, B. L. & Dizon, A. E. 1999. First policy then science: why a management unit based soley on genetic criteria cannot work. *Molecular Ecology*. (8): S11-S16.

Temminick, C. J. 1853. *Esquisses Zoologiques sur la Côte de Guineé*. E. J. Brill, Leiden.

Tinley, K. L. 1985. *Coastal Dunes of South Africa. South African National Scientific Programmes Report* No. **109**.

Tosi, A. J., Disotell, T. R., Morales, J. C. & Melnick, D. J. 2005. Cercopithecine Y-chromosome data provide a test of competing morphological evolutionary hypotheses. *Molecular Phylogenetics and Evolution* **27**: 510-521.

Tyson, P. D. 1986. *Climate Change and Variability in Southern Africa*. Oxford University Press, Cape Town.

Van der Kuyl, A. C., Dekker J. T. & Goudsmit, J. 2000. Primate Genus *Miopithecus*: evidence for the existence of species and subspecies of dwarf guenons based on cellular and endogenous viral sequences. *Molecular Phylogenetics and Evolution.* **14**: 403-413.

Van der kuyl, A. C., Kuiken, C. L., Dekker, J. T. and Goudsmit, J. 1995. Phylogeny of African monkeys based upon mitochondrial 12S rRNA sequences. *Journal of Molecular Evolution* **40**: 173-180.

Verheyen, W.N. 1962. *Contribution à la Craniologie comparée des Primates: les Genres Colobus Illiger 1811 et Cercopithecus Linné 1758.* Musée Royal de l'Afrique Centrale, Tervuren.

Waples, R. S. 1995. Evolutionarily significant units and the conservation of biological diversity under the Endangered Species Act. In: *Evolution and the Aquatic Ecosystem: Defining Unique Units in Population Conservation*, ed. J. L. Nielsen and G. A. Powers. Symposium 17. *American Fisheries Society*, Bethesda, Maryland.

Waples, R. S. 1998. Evolutionarily significant units, distinct population segments, and the endangered species act: reply to Pennock, D. S. & Dimmick, W. W. *Conservation Biology* **12**: 718-721.

White, F. 1981. The history of the Afromontane archipelago and the scientific need for its conservation. *African Journal of Ecology* **19**: 33-54.

Wolf, J. 1822. *Abbildungen und Beschreibungen merkwurdiger naturgeschichtlicher Gegenstände.* Nürnberg 2.

Wood, B. & Richmond, B. G. 2000. Human evolution: taxonomy and paleobiology. *Journal of Anatomy* **197**: 19–60.

Wu, C. I. & Hollocher, H. 1998. Subtle is nature: the genetics of species differentiation and speciation. *Endless Forms*: *Species and Speciation* (2): 879.

Zimmerman, E. 1990.Differentiation of vocalization in bushbabies (Galaginae, Prosimia, Primates) and the significance for assessing phylogenetic relationships. *Journal of Zoological Systematics and Evolutionary Research*. 28(3): 217-239.

Zimmerman, E., Bearder, S. K., Doyle, G. A. & Anderson, A. 1998. Variations in vocal patterns of Senegal and South African lesser bushbabies and their implications for taxonomic relationships. *Folia Primatologica* **51**:87-105.

Appendix 1. Standard body measurements recorded from skins housed in the Natural History Museum of Zimbabwe in Bulawayo and from Hogsback village and forest *C. a. labiatus* troops.

Sex	**Age**	**Head-Body length (mm)**	**Tail length (mm)**	**Hindfoot length (mm)**	**Ear length (mm)**	**Species and other information**	**Locality**
M	A	460	770	160	55	*C. mitis* Overall length= 1230 mm	Zimbabwe
M	A	570	800	160	35	*C. mitis* Shank length=160 mm and Overall length= 1340 mm	Zim
F	A	450	673	132	45	*C. mitis* Overall length=1123 mm	Zim
F	A	480	720	138	35	*C. mitis* Overall length=1200mm, **Weight=** 10 Ibs ~ **4.5 kg**	Zim
F	A	482	628	126	41	*C. mitis* **Weight=** 8 Ibs ~**3.6 kg**	Zim
M	A	390	620	135	40	*C. mitis*	Zim
F	A	484	726	135	32	*C. mitis* Overall length=1210	Zim

F	A	500	700	142	35	*C. mitis* Overall length=1200	Zim
M	A	520	600	153	33	*C. mitis* Overall length=1120	Zim
M	A	625	820	178	43	*C. mitis* Overall length= 1445, **Weight**= 22 lbs ~ **10 kg**	Zim
F	A	480	695	160	40	*C. mitis* Overall length 1175, **Weight**=10 lbs~ **4.5 kg**	Zim
	A	475	695	143	43	*C. mitis* Overall length=1170, **Weight**=11 lbs~ **5kg**	Zim
F	A	452	460	131	40	*C. mitis* Overall length=912, **Weight=**9 lbs ~**4kg**	Zim
M	A	405	562	123	34	*C. mitis* Overall length=967, **Weight=** 6lbs~**3kg**	Zim
M	A	440	490	120	40	*C. mitis leucampyx labiatus*	
F	A	485	555	133	35	*C. mitis*	Zim
F	A	520	820	164	39	*C. mitis*	Zim
M	A	585	790	170	40	*C. mitis*	Zim

F	A	526	670	145	35	*C. mitis* **W**=11 lbs~**5kg**	Zim
M	A	602	813	168	38	*C. mitis*	
M	A	530	793	166	35	*C. mitis*	Zim
M	A	600	700	155	40	*C. mitis*	Zim
F	A	415	600	135	38	*C. m. opistosticus* **W**=8Ibs **3.6kg**	Zim
F	A	469	674	139	31	*C. mitis opistosthicus* **W**= 12 Ibs~**5.4kg**	Zim
F	A	395	625	128	37	*C. mitis opisthostictus* **W**= 10 Ibs~**4.5kg**	
F	A	483	686		25	*C. m. opisthostictus*	Zim
F	A	483	584	381		*C. m. moloneyi*	Zim
F	A	459	646	141	33	*C. m. opisthostictus* **W**= 11 Ibs~**5kg**	Zim
M	A	502	761	162	35	*C. m. opisthostictus* **W**=15 Ibs~ **7kg**	Zim
M	A	510	830	145	30	*C. nictitans*	Zim
F	A	370	585	150	29	*C.nictitans* **W**=4 Ibs~ **2kg**	Zim
F	A	480	750	145	38	*C. m. erythrachus* W= 11 Ibs~ **5kg**	Zim

M	A	550	814	153	42	*C. albogularis* **W= 7kg**	Zim
F	A	448	655	129	38	*C. albogularis* **W= 3 kg**	Zim
F	A	485	678	133	39	*C. albogularis*	Zim
F	A	441	644	147	35	*C. albogularis* **W= 4kg**	Zim
	A		820	164	38	*C. m. moloneyi*	Malawi
Hogsback Village troop	**Sex**	**Est. age group**	**Weight (kg)**	**Head to body (mm)**	**Tail length (mm)**	**Hind foot (no claw)**	**Ear length (mm)**
1	F	A	4	450	600	128	30
2	M	A	8	545	705	155	40
3	F	A	4	455	565	133	38
6	F	A	4	450	580	132	36

7	F	A	4	440	610	138	38
8	F	A	4	430	555	125	34
9	F	A	4	460	560	130	34
13	M	A	6.2	510	750	159	36
14	M	A	6.4	575	675	155	37
15	F	A	4.2	470	580	130	38
16	F	A	4.3	460	460	130	32
Hogsback Forest Troop	**Sex**	**Est Age Group**	**Weight (kg)**	**Head-Body length (mm)**	**Tail Length (mm)**	**Hind Foot (no claw)**	**Ear Length (mm)**

17	F	A	3	360	495	120	41
18	F	A	4	455	540	130	37
19	F	A	5	460	460	120	38
6	M	A	7	520	665	150	38

Appendix 2. Museum skull specimens used in my study

Museum	**Accession number**	**Sex**	**Age**	**Species**	**Locality**
Amathole	14913	F	A	*C. a. labiatus*	Frankfort
Amathole	10723	M	A	*C. a. moloneyi*	Saisi valley N.Rodesia
Amathole	10620	F	A	*C. a. moloneyi*	Sunzie Hill, N.Rhodesia
Amathole	10663	F	A	*C. a. labiatus*	Frankfort,KWT
Amathole	10655	F	A	*C. a. labiatus*	Frankfort,KWT
Transvaal	7726	F	A	*C. a. erythrachus*	Vumba Zimbabwe
Amathole	10641	F	A	*C. a. labiatus*	Frankfort,Pierie
Amathole	13309	F	A	*C. a. labiatus*	Mt.Coke KWT
Amathole	11546	F	A	*C. a. moloneyi*	Nchisi Mt. Nyasaland
Amathole	10910	F	A	*C. a. erythrachus*	Maringaskraal Mozambique
Amathole	13993	F	A	*C. a. labiatus*	Peddie, Eastern Cape
Amathole	10653	F	A	*C. a. labiatus*	Frankfort,KWT
Amathole	10627	F	A	*C. a. moloneyi*	Itinga Mts N.Rhodesia
Amathole	11548	F	A	*C. a. nyasae*	Nyasaland, Cholo Mt. Slopes
Amathole	10623	F	A	*C. a. moloneyi*	Kalambo River N. Rhodesia
Amathole	11448	F	A	*C. a. erythrachus*	Inyanga Zimbabwe
Amathole	13000	M	A	*C. a. labiatus*	Frankfort,KWT
Amathole	10626	F	A	*C. a. moloneyi*	Mufinga Mts. N. Rhodesia
Transvaal	30718	M	A	*C. a. erythrachus*	Natal Tonggoland
Transvaal	7725	F	A	*C. a. erythrachus*	Mtn selinda S. Rhodesia
Amathole	11549	F	A	*C. a. nyasae*	Nyasaland, Cholo Mt. Slopes
Amathole	18908	M	A	*C. a. labiatus*	Pirie Forest,KWT
Amathole	10650	M	A	*C. a. labiatus*	Frankfort,KWT
Amathole	10639	M	A	*C. a. labiatus*	Frankfort,Pierie
Amathole	10647	M	A	*C. a. labiatus*	Frankfort,Pierie Forest

Amathole	10625	M	A	*C. a. moloneyi*	Mufinga Mts. N.Rhodesia
Transvaal	25705	M	A	*C. a. erythrachus*	Natal Ubombo Rsrv Stn Lake Sibiya
Transvaal	25768	M	A	*C. a. erythrachus*	Natal Ubombo Rsrv Stn Lake Sibiya
Transvaal	40168	M	A	*C. a. erythrachus*	Zululand Hlabisa Dist.Cape Vidal
Amathole	11552	M	A	*C. a. nyasae*	S.Nyasa land,Cholo Mt. slopes
Transvaal	41900	M	A	*C. a. erythrachus*	Natal Karkloof Bracco PMB
Bulawayo	67713	F	A	*C. albogularis*	Zinave Mozambique
Bulawayo	67714	F	A	*C. albogularis*	Zinave Mozambique
Bulawayo	28566	F	A	*C. albogularis*	Rhodesia Vumba National Park
Bulawayo	2630	F	A	*C. mitis opisthostictus*	Zambia
Bulawayo	67854	F	A	*C. albogularis*	Pungwe Bridge Honde valley Rhod
Bulawayo	7090	F	A	*C. albogularis*	Rhodesia Lower Pungwe Holdenby
Bulawayo	26609	F	A	*C. albogularis*	Rhodesia Chimani-chimani
Bulawayo	5237	F	A	*C. albogularis*	South Rhodesia Haroni
Bulawayo	16524	F	A	*C. a. moloneyi*	Zambia
Bulawayo	28190	F	A	*C. m. opisthostictus*	Zambia
Bulawayo	10038	F	A	*C. m. opisthostictus*	Zambia
Bulawayo	2619	F	A	*C. m. opisthostictus*	Zambia
Bulawayo	67892	M	A	*C. a. moloneyi*	Misuku Malawi
Bulawayo	67873	F	A	*C. albogularis*	Haroni Rusitu confluence Zimbabwe
Bulawayo	2627	M	A	*C. a. opisthostictus*	Zambia
Bulawayo	67875	F	A	*C. mitis*	Glen Eagles Nyanga Rhod
Bulawayo	5771	F	A	*C. a. opisthostictus*	Zambia
Bulawayo	5236	F	A	*C. albogularis*	South Rhodesia Haroni
Bulawayo	50147	F	A	*C. albogularis*	National Parks
Bulawayo	67881	F	A	*C. albogularis*	Chitengo Mozambique
Bulawayo	67882	F	A	*C. albogularis*	Mozambique Gorongoza National Park

Bulawayo	4016	F	A	*C. m. opisthostictus*	Zambia
Bulawayo	8311	M	A	*C. m. opisthostictus*	Zambia
Bulawayo	67874	F	A	*C. albogularis*	Mountain Selinda Zim
Bulawayo	67866	M	A	*C. albogularis*	Vumba Zimbabwe
Bulawayo	22653	M	A	*C. m. opisthostictus*	N. Rhod: Zambia
Bulawayo	7095	F	A	*C. albogularis*	South Rhodesia Haroni
Bulawayo	67869	M	A	*C. albogularis*	Vumba National Park Zim
Bulawayo	67880	M	A	*C. albogularis*	Mozambique Gorongoza National Park
Bulawayo	20354	M	A	*C. a. moloneyi*	Muchinga Escarpment Zambia
Bulawayo	67871	M	A	*C. albogularis*	Hermitage Vumba Zim
Bulawayo	56782	M	A	*C. albogularis*	Wiermouth Mutare Zim
Bulawayo	67856	M	A	*C. albogularis*	Vumba National Park, Zim
Bulawayo	67883	M	A	*C. a. francenscae*	Malawi

Appendix 3. Scores for DFA1. Only one specimen (represented in bold) was apparently misclassified in this analysis.

Categories	Species/Subspecies	Misclassification	Score 1	Score 2
e	*C. a. erythrarchus*	0	-6.94432	-1.10339
e	*C. a. erythrarchus*	0	-6.31516	-1.79087
e	*C. a. erythrarchus*	0	-8.39272	-1.99825
e	*C. a. erythrarchus*	0	-5.29398	-1.91585
e	*C. a. erythrarchus*	0	-5.64143	-2.6732
e	*C. a. erythrarchus*	0	-7.39547	-2.42109
e	*C. a. erythrarchus*	0	-6.93097	-1.13949
e	*C. a. erythrarchus*	0	-6.87657	-2.0333
e	*C. a. erythrarchus*	0	-8.01714	-2.42735
l	*C. a. labiatus*	0	-5.27615	0.412893
l	*C. a. labiatus*	0	-6.26584	1.078335
l	*C. a. labiatus*	0	-5.42134	2.138952
l	*C. a. labiatus*	0	-5.09974	0.761017
l	*C. a. labiatus*	0	-5.65969	3.060801
l	*C. a. labiatus*	0	-4.82724	1.522981
l	*C. a. labiatus*	0	-5.61857	2.537187
l	*C. a. labiatus*	0	-6.28239	1.284747
l	*C. a. labiatus*	0	-5.68058	1.048596
l	*C. a. labiatus*	0	-5.59007	1.784258
l	*C. a. labiatus*	0	-5.84815	1.747577
s	*C. a. moloneyi*	0	-2.13114	0.381699
s	*C. a. moloneyi*	**1**	**-4.45249**	**-1.8131**
s	*C. a. moloneyi*	0	-5.19752	1.392301
s	*C. a. nyasae*	0	-4.72192	0.527359
s	*C. a. moloneyi*	0	-4.56309	-0.01173
s	*C. a. moloneyi*	0	-4.57704	1.14431
s	*C. a. nyasae*	0	-4.99501	-0.17112
s	*C. a. moloneyi*	0	-3.37405	0.591351
s	*C. a. nyasae*	0	-4.09719	0.578167
b	*C. albogularis*	0	4.35922	1.527549
b	*C. albogularis*	0	3.866423	-0.41965
b	*C. albogularis*	0	6.810867	-0.65648
b	*C. m. opisthostictus*	0	4.107371	1.867588
b	*C. albogularis*	0	5.507064	1.003481
b	*C. albogularis*	0	4.543826	0.321506
b	*C. albogularis*	0	4.722061	-0.04565
b	*C. albogularis*	0	4.605969	-0.35121
b	*C. a. moloneyi*	0	3.588637	-0.9536
b	*C. m. opisthostictus*	0	5.875159	-0.66718
b	*C. m. opisthostictus*	0	4.211557	-1.9066
b	*C. m. opisthostictus*	0	4.429598	0.307321
b	*C. a. moloneyi*	0	5.001481	0.468043

b	*C. albogularis*	0	5.098262	0.246032
b	*C. m. opisthostictus*	0	2.48841	1.173572
b	*C. albogularis*	0	4.677452	-0.49139
b	*C. m. opisthostictus*	0	4.804886	0.394395
b	*C. a .albogularis*	0	5.864319	-0.06803
b	*C. albogularis*	0	2.270381	0.996278
b	*C. albogularis*	0	5.525423	-0.21271
b	*C. albogularis*	0	5.60944	-0.88127
b	*C. m. opisthostictus*	0	5.027192	2.309335
b	*C. m. opisthostictus*	0	5.71099	0.512066
b	*C. albogularis*	0	3.414643	-1.0823
b	*C. albogularis*	0	4.499374	-1.15598
b	*C. m. opisthostictus*	0	5.026605	1.347752
b	*C. albogularis*	0	6.527917	0.297457
b	*C. albogularis*	0	2.616633	-3.29052
b	*C. albogularis*	0	3.220741	-0.68492
b	*C. a. moloneyi*	0	6.440706	-1.17449
b	*C. albogularis*	0	6.161336	-2.03117
b	*C. albogularis*	0	5.46232	0.039386
b	*C. albogularis*	0	4.666641	0.471331
b	*C. a. francescae*	0	4.744065	0.296257

Appendix 4. Scores for DFA2

Category	Subspecies	Misclassification	Score 1	Score 2
e	*C. a. erythrarchus*	0	2.813404	-1.91081
e	*C. a. erythrarchus*	0	3.437616	-4.04475
e	*C. a. erythrarchus*	0	4.44108	-3.33461
e	*C. a. erythrarchus*	0	5.880004	-0.13395
e	*C. a. erythrarchus*	0	6.032721	-0.32094
e	*C. a. erythrarchus*	0	6.019502	-3.61145
e	*C. a. erythrarchus*	0	4.393987	-3.17995
e	*C. a. erythrarchus*	0	4.895803	-1.31609
e	*C. a. erythrarchus*	0	4.436384	-2.56859
l	*C. a. labiatus*	0	-4.20091	-2.22571
l	*C. a. labiatus*	0	-2.35814	-1.87569
l	*C. a. labiatus*	0	-5.38542	-0.20126
l	*C. a. labiatus*	0	-3.08516	-2.07609
l	*C. a. labiatus*	0	-4.94463	-1.82877
l	*C. a. labiatus*	0	-5.06363	-0.70199
l	*C. a. labiatus*	0	-4.81573	-0.68599
l	*C. a. labiatus*	0	-4.59836	-1.03339
l	*C. a. labiatus*	0	-4.45951	-2.01696
l	*C. a. labiatus*	0	-3.89925	-2.28181
l	*C. a. labiatus*	0	-4.05142	-3.10384
o	*C. a. moloneyi*	0	-0.67564	4.575649
o	*C. a. moloneyi*	**1**	1.171105	4.173553
o	*C. a. maloneyi*	0	1.011572	5.195339
o	*C. a. nyasae*	0	-0.33295	3.917021
o	*C. a. moloneyi*	0	2.108104	3.335071
o	*C. a. moloneyi*	0	0.181233	4.259039
o	*C. a. nyasae*	0	1.469744	4.200924
o	*C. a. moloneyi*	0	-0.5456	4.231851
o	*C. a. nyasae*	0	0.124114	4.564178

Appendix 5. Animals captured for genetic analysis.

Forest Troop	Village Troop
F_1	V_1
F_2	V_2
F_3	V_3
F_4	V_4
F_5	V_5
F_6	V_6
F_7	V_7
F_8	V_8
	V_9
	V_{10}
	V_{15}
	V_{17}
	V_{22}

Appendix 6. Skull specimen localities and their co-ordinates (converted into degrees decimal) used in my study

Species	**Locality**	**Country**	**Degrees Decimal Latitude**	**Degrees Decimal Longitude**
C. albogularis francescae	Malawi	MAL	-13.5	34
C. albogularis moloneyi	Misuku, Malawi	MAL	-9.666666667	33.51666667
C. a. erythrarchus	Maringaskraal, Mozambique	MOZ	-18.25	35
C. albogularis	Mozambique, Gorongoza National Park	MOZ	-18.76611111	34.5
C. albogularis	Chitengo, Mozambique	MOZ	-18.97916667	34.35305556
C. albogularis	Mozambique, Gorongoza National Park	MOZ	-18.76611111	34.5
C. albogularis	Zinave, Mozambique	MOZ	-21.43666667	33.85027778
C. albogularis	Zinave, Mozambique	MOZ	-21.43666667	33.85027778
C. a. erythrarchus	Natal Karkloof Bracco Pietermaritzburg	SA	-29.45	30.31666667
C. a. erythrarchus	Natal Tongoland	SA	-27.16666667	32.33333333
C. a. erythrarchus	Natal Ubombo Reserve Station Lake Sibiya	SA	-29	30
C. a. erythrarchus	Natal Ubombo Rsrv Stn Lake Sibiya	SA	-29	30
C. a. erythrarchus	Zululand Hlabisa Dist.Cape Vidal	SA	-28.13333333	31.86666667
C. albogularis labiatus	Frankfort, King William's Town (KWT)	SA	-32.73333333	27.46666667
C. a. labiatus	Frankfort,KWT	SA	-32.73333333	27.46666667
C. a. labiatus	Frankfort,KWT	SA	-32.73333333	27.46666667
C. a. labiatus	Frankfort,KWT	SA	-32.73333333	27.46666667

C. a. labiatus	Frankfort,KWT	SA	-32.73333333	27.46666667
C. a. labiatus	Frankfort,KWT	SA	-32.73333333	27.46666667
C. a. labiatus	Frankfort,Pierie	SA	-32.73333333	27.46666667
C. a. labiatus	Frankfort,Pierie	SA	-32.73333333	27.46666667
C. a. labiatus	Frankfort,Pierie Forest	SA	-32.73333333	27.46666667
C. a. labiatus	Mt.Coke KWT	SA	-32.96666667	27.43333333
C. a. labiatus	Peddie, Eastern Cape	SA	-33.2	27.11666667
C. a. labiatus	Pirie Forest,KWT	SA	-32.73333333	27.46666667
C. mitis opisthostictus	N. Rhodesia, Zambia	ZAM	-15	30
C. m. opisthostictus	Zambia	ZAM	-15	30
C. m. opisthostictus	Zambia	ZAM	-15	30
C. m. opisthostictus	Zambia	ZAM	-15	30
C. m. opisthostictus	Zambia	ZAM	-15	30
SS: C. a. moloneyi	Zambia	ZAM	-15	30
SS: C. mitis opisthostictus	Zambia	ZAM	-15	30
SS: C. m. opisthostictus	Zambia	ZAM	-15	30
SS: C. m. opisthostictus	Zambia	ZAM	-15	30
SS: C. m. opisthostictus	Zambia	ZAM	-15	30
C. albogularis	National Parks	ZIM	-19.08333333	32.75
C. albogularis	Wiermouth Mutare Zimbabwe	ZIM	-18.96666667	32.66666667
C. a. moloneyi	Muchinga Escarpment, Zambia	ZAM	-13.66666667	31
C. albogularis	Vumba National Park, Zim	ZIM	-19.08333333	32.75
C. a. erythrarchus	Nyanga, Zimbabwe	ZIM	-18.21666667	32.75
C. a. erythrarchus	Mtn selinda S.Rhodesia	Moz	-20.45	32.71666667
C. a. erythrarchus	Vumba, Zimbabwe	ZIM	-19.08333333	32.75

C. a. moloneyi	Itinga Mts N.Rhodesia	ZAM	-17.58333333	26.76666667
C. a. moloneyi	Kalambo River N.Rhodesia	ZAM	-8.6	31.18333333
C. a. moloneyi	Mafinga Mts. N.Rhodesia	ZAM	-10	33.31666667
C. a. moloneyi	Mafinga Mts. N.Rhodesia	ZAM	-10	33.31666667
C. a. moloneyi	Nchisi Mt. Nyasaland	Mal	-13.31666667	34.05
C. a. moloneyi	Saisi valley N.Rhodesia	ZAM	-15.28333333	23.03333333
C. a. moloneyi	Senga Hill, N.Rhodesia	ZAM	-9.35	31.21666667
C. a. nyasae	Nyasaland,Cholo Mt. Slopes	Mal	-16.05	35.03333333
C. a. nyasae	Nyasaland,Cholo Mt. Slopes	Mal	-16.05	35.03333333
C. a. nyasae	S.Nyasaland,Cholo Mt. slopes	Mal	-16.05	35.03333333
C. albogularis	Hermitage Vumba Zimbabwe	ZIM	-19.08333333	32.75
C. albogularis	Mountain Selinda Zimbabwe	MOZ	-20.45	32.71666667
C. albogularis	Rhodesia Chimanimani	ZIM	-19.8	32.86666667
C. albogularis	South Rhodesia(Mozambique) Haroni	MOZ	-20.01666667	33.01666667
C. albogularis	South Rhodesia(Mozambique) Haroni	MOZ	-20.01666667	33.01666667
C. albogularis	South Rhodesia(Mozambique) Haroni	MOZ	-20.01666667	33.01666667
C. albogularis	Vumba Zimbabwe	ZIM	-19.08333333	32.75
C. albogularis	Haroni Rusitu confluence Zimbabwe	ZIM	-20.05861111	32.855

C. albogularis	Vumba National Park Zimbabwe	ZIM	-19.08333333	32.75
C. albogularis	Gleneagles Nyanga Rhodesia	ZIM	-18.3	32.85
C. albogularis	Rhodesia Lower Pungwe Honde valley	ZIM	-18.42194444	32.78111111
C. albogularis	Rhodesia Vumba National Park	ZIM	-19.08333333	32.75
C. albogularis	Pungwe Bridge Honde valley Rhodesia	ZIM	-18.42194444	32.78111111