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Phylogeography and impact of hybridization on the evolution of African green monkeys (*Chlorocebus* Gray, 1870)

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Tanja Haus

aus

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Referentin: Prof. Dr. Julia Fischer

Korreferent: Prof. Dr. Eckhard Heymann

Tag der mündlichen Prüfung:

Warren H. Wagner once said, “We used to make fun of Edgar Anderson by saying that he was finding hybrids under every bush. Then we realized that even the bushes were hybrids.” (relayed by Michael Arnold in Abbott et al., 2013, p. 241).

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SUMMARY

The evolution of the current global biodiversity has been profoundly influenced by climatic and environmental changes over the past million years. While there is an obvious impact of Quaternary climate changes and glacial cycles on Northern Hemisphere biota, the influence of these climate conditions on (sub-) tropical regions, especially on the African savannah biome, is less clear. Nonetheless, environmental changes in tropical and non-tropical regions produced a large number of current hybrid zones, where previously isolated populations came into secondary contact. Although the evolutionary potential of hybridization has become generally accepted, many open questions remain and our understanding of the role of hybridization within the evolution of animals is by far not complete.

The distribution of African green monkeys of the genus *Chlorocebus* almost reflects the current extension of the African savannah biome and, based on field observations and museum material, hybridization is supposed to occur in most areas where ranges of two of the six recognized parapatric species come into contact. Therefore, in my thesis I used African green monkeys as a model to analyse the role of hybridization in the evolution of primates and animals in general, and to examine major evolutionary trends in extant African savannah mammals. First I analysed complete cytochrome *b* (cyt *b*) gene sequences of samples representing all species and most of the genus' wide range to obtain elementary information on the mitochondrial DNA (mtDNA) diversity and distribution of African green monkeys. Second I used sequence variations of two Y-chromosomal loci, a fragment of the sex determining region (SRY) and the last intron of the Zinc finger (ZFY), to test for potential ancient hybridization events, and third I analysed additional mitochondrial markers of samples from each previously obtained mtDNA clade to increase genetic information for spatial and temporal phylogeographic reconstructions. Finally, I compared obtained phylogeographic patterns of African green monkeys to phylogeographies of three other widely distributed savannah mammal genera including baboons (*Papio*), warthogs (*Phacochoerus*) and hartebeests (*Alcelaphus*).

Analyses of mtDNA revealed nine distinct clades that do not reflect any previously suggested taxonomy of African green monkeys. Several para- and polyphyletic relationships caused by discordance of mtDNA and morphotype distribution provide evidence for on-going introgressive hybridization in contact zones of all species except for species in West Africa. Moreover, mitochondrial data indicate the occurrence of possible ancient introgressive hybridization beyond current contact zones, and Y-chromosomal analyses further support this assumption within the species *C. pygerythrus* in East Africa. Here male biased ancient introgression and subsequent nuclear swamping most likely led to the cytonuclear extinction of a former taxon. However, polyphyletic relationships within *C. tantalus* could not be explained by ancient introgression, and Y-chromosomal data rather support the hypothesis that two morphologically cryptic taxa exist (a western and an eastern) within the range of *C. tantalus*. This is the first genetic indication of a possible new species. The absence of hybridization among *C. sabaeus* and the western form of *C. tantalus* in Ghana and Burkina Faso is also shown by concordant distributions of Y-chromosomal haplotypes and morphotypes assuming that the Volta River and its tributaries represent geographic barriers throughout their courses and not only in the South as it was previously supposed.

The phylogeography of African green monkeys indicates a complex evolutionary history with an origin of the genus in West Africa around 2.46 mya, a subsequent immigration into Southern Africa and two independent colonisations of Northeast Africa from the West and from the South, respectively. The comparison of the phylogeography of savannah mammals revealed no concordant pattern of divergence time estimates among primate and ungulate genera and divergences occurred during regional arid as well as humid periods. Changes in population sizes over the past 500,000 years show dissimilar patterns between primate and ungulate genera, which most probably reflect different ecological adaptations and habitat preferences although all four genera are predominantly savannah living. Moreover, most of the changes in population sizes of all genera combined did not coincide with the Last Interglacial and the Last Glacial Maximum supporting the influence of regional African climate variations.

In conclusion, African green monkeys evolved in the beginning of the Early Pleistocene and Quaternary climatic changes likely caused recurrent range retractions

and extensions, which in turn led repeatedly to the formation of secondary contact zones and to widespread introgressive hybridization within the genus. The cytonuclear extinction of former taxa or populations as a result of male-biased introgression and nuclear swamping accentuate the potential role of hybridization within the evolution of primates and animals in general. Major divergences within savannah mammals were most probably induced by both warm-humid as well as cold-arid climate periods, which were rather triggered by more regional African climate variations than by Northern Hemisphere glacial cycles. The results of my thesis indicate that it is essential for phylogeographic studies to analyse both maternal and paternal inherited markers to obtain a more complete picture of evolutionary patterns, potential hybridization, and of genetic and species diversity.

ZUSAMMENFASSUNG

Die Evolution der heutigen globalen Diversität wurde in den letzten Millionen Jahren insbesondere durch klimatische Schwankungen und entsprechende Veränderungen biologischer Lebensräume beeinflusst. Besonders deutlich ist der Einfluss von Glazialen und Interglazialen auf die Evolution von Organismen der Nördlichen Hemisphäre. Weniger klar hingegen ist, inwiefern sich diese klimatischen Verhältnisse auf (sub-) tropische Regionen ausgewirkt haben, insbesondere auf das Afrikanische Savannen Biom. Unabhängig davon, haben Umweltveränderungen in tropischen und nicht-tropischen Gebieten zur Entstehung vieler der gegenwärtigen Hybridzonen geführt, in denen zuvor geographisch separierte Populationen in sekundären Kontakt gekommen sind. Obwohl Hybridisierung im Tierreich inzwischen nicht mehr als ein rares Phänomen betrachtet wird, ist das tatsächliche Ausmaß und die Bedeutung von Hybridisierung in der Evolution von Tieren noch lange nicht vollständig geklärt.

Die Verbreitung Grüner Meerkatzen der Gattung *Chlorocebus* reflektiert nahezu die Ausdehnung Afrikanischer Savannengebiete und basierend auf Beobachtungen im Freiland sowie auf morphologischen Merkmalen von Museumsexemplaren, hybridisieren die meisten der sechs anerkannten parapatrischen Arten in ihren jeweiligen Kontaktzonen. Aufgrund dieser Eigenschaften habe ich in meiner Doktorarbeit Grüne Meerkatzen als Modellsystem genutzt, um zum einen die Bedeutung von Hybridisierung in der Evolution von Primaten und Tieren im Allgemeinen zu untersuchen, und zum anderen, um wesentliche Trends in der Evolution von Savannensäugetieren zu analysieren. Um in einem ersten Schritt grundlegende Informationen über die mitochondriale Diversität und Verbreitung der Grünen Meerkatzen zu erlangen, generierte ich vollständige Cytochrom b Sequenzen von Proben, die alle sechs Arten der Gattung und große Teile der gesamten Verbreitung Grüner Meerkatzen repräsentierten. Des Weiteren nutzte ich Sequenz-Informationen zweier Y-chromosomaler Loci, ein Fragment der *sex determining region* (SRY) und das letzte Intron des *Zinc finger* (ZFY), um eventuell zeitlich zurückliegende Hybridisierungsereignisse nachzuweisen. Um räumliche sowie zeitliche phylogeographische Muster zu rekonstruieren, habe ich basierend auf den bisher

gewonnen Daten weitere mitochondriale Marker von selektiven Proben aller mitochondrialer Kladen sequenziert. Abschließend habe ich die Phylogeographie Grüner Meerkatzen mit den Phylogeographien dreier anderer weit verbreiteter Savannensäugetier-Gattungen verglichen, mit Pavianen (*Papio*), Warzenschweinen (*Phacochoerus*), und Kuhantilopen (*Alcelaphus*).

Meine Analysen der mitochondrialen Daten lassen neun klar abgegrenzte Kladen erkennen, die keiner bisher vorgeschlagenen Taxonomie entsprechen. Zahlreiche para- und polyphyletische Beziehungen, verursacht durch nicht übereinstimmende Verbreitungsmuster mitochondrialer Kladen und morphologischer Merkmale, liefern Hinweise auf anhaltende introgressive Hybridisierung in den Kontaktzonen aller Arten, mit Ausnahme der beiden westafrikanischen Arten. Darüber hinaus weisen die Ergebnisse der mitochondrialen Analysen auf potentiell vergangene introgressive Hybridisierungsereignisse hin, die geographisch nicht in Gebiete gegenwärtiger Kontaktzonen fallen. Dies kann im Falle von *C. pygerythrus* in Ostafrika anhand der Y-chromosomalen Daten bestätigt werden. Männchen basierte Introgression und *nuclear swamping* haben hier offensichtlich zu dem zytonukleären Aussterben eines historischen Taxons geführt. Aber nicht alle diskordante Muster in der mitochondrialen Phylogenie sind Anzeichen für zurückliegende introgressive Hybridisierung. Innerhalb der Verbreitung von *C. tantalus* weisen sowohl mitochondriale als auch Y-chromsomale Daten auf zwei klar separierte und morphologisch kryptische Taxa hin (ein westliches und ein östliches Taxon), ein Befund der möglicherweise auf das Vorhandensein einer neuen Grünen Meerkatzenart hindeutet. In Übereinstimmung mit mitochondrialen Ergebnissen weisen Y-chromosomale Daten ebenfalls keine Anzeichen für Hybridisierung zwischen *C. sabaeus* und der westlichen *C. tantalus*-Form in Westafrika auf. Entgegen früherer Annahmen stellt der Volta Fluss und seine nördlicheren Zuflüsse offenbar auf gesamter Länge eine geographische Barriere dar und nicht wie vorher angenommen nur im südlicheren Teil des Flussverlaufs.

Die Phylogeographie Grüner Meerkatzen weist somit auf eine komplexe evolutionäre Geschichte hin. Aufgrund der phylogenetischen Rekonstruktionen und Datierungen gehe ich von einem Westafrikanischen Ursprung der Gattung vor ca. 2,46 Millionen Jahren aus. Des Weiteren liefert die Phylogenie Hinweise auf eine erst später

folgende Ausbreitung bis nach Südafrika und auf zwei zeitlich getrennte Besiedelungen nordöstlicher Regionen, eine vom Westen und eine von eher südlicheren Regionen aus. Im Vergleich zu den anderen Savannensäugetieren können keine zeitlichen Übereinstimmungen in Aufspaltungsmustern gefunden werden, weder im Vergleich zwischen den Primaten noch zwischen den Ungulaten. Zudem fallen Aufspaltungen innerhalb der Gattungen zeitlich sowohl mit kalt-ariden als auch mit warm-humiden Perioden zusammen. Veränderungen in Populationsgrößen innerhalb der letzten 500.000 Jahre zeigen unterschiedliche Muster zwischen Primaten und Ungulaten, die wahrscheinlich mit verschiedenen ökologischen Anpassungen und Habitat Präferenzen zu erklären sind. Da außerdem keine klaren Zusammenhänge zwischen dem zeitlichen Auftreten von Populationsschwankungen und dem letzten Interglazialem bzw. Glazialen Maximum gefunden werden können, scheint ein Einfluss von regional geprägtem Klima in Afrika wahrscheinlicher.

Zusammengefasst hat die Evolution Grüner Meerkatzen im frühen Pleistozän begonnen und klimatische Schwankungen im Quartär haben vermutlich zu wiederkehrenden Veränderungen in der Ausbreitung geführt. Diese begünstigte die Entstehung von sekundären Kontaktzonen und führte zu weit verbreiteter introgressiver Hybridisierung innerhalb der Gattung. Das zytonukleäre Aussterben ehemaliger Taxa oder Populationen als Ergebnis lang anhaltender introgressiver Hybridisierung und darauffolgendem *nuclear swamping* machen zudem den potentiellen Einfluss von Hybridisierung in der Evolution von Primaten und Tieren im Allgemeinen deutlich. Die Phylogeographien der Savannensäugetiere zeigen, dass Aufspaltungen innerhalb der Gattungen sehr wahrscheinlich durch ausgeprägt humide wie auch aride Bedingungen begünstigt wurden, die eher durch regionale Klimatische Veränderungen in Afrika zu erklären sind als durch Glaziale Zyklen und Klimaschwankungen der Nördlichen Hemisphäre. Die Ergebnisse meiner Doktorarbeit betonen die Notwendigkeit der Analyse von sowohl mütterlich als auch väterlich vererbten Markern in phylogeographischen Studien, um ein möglichst vollständiges Bild von evolutionären Prozessen, Hybridisierung, sowie von genetischer und taxonomischer Vielfalt zu erlangen.

Chapter 1 General Introduction

Why are certain organisms where they are and how and when did they get there? These questions play a central role in phylogeographic research. With the advance of molecular genetic sequence analysis and its application to various taxonomic groups "striking patterns were being uncovered in the spatial arrangements of mitochondrial DNA lineages. In other words genealogy and geography seemed to be connected." (Avise, 2009, p. 3). This connection is used in current phylogeographic studies, which consider combinations of phylogenetic, population genetic and geographic approaches to untangle evolutionary histories of organisms (Avise, 1998). An important number of the current global biodiversity is the result of divergent adaptation in response to heterogeneous and changing environments. Central factors which produce pronounced environmental shifts and heterogeneity are tectonic and climatic changes, and it is known that the evolution among mammals has been profoundly influenced by climatic changes over the past million years (Janis, 1993; Rundle & Nosil, 2005; Seehausen et al., 2008). This especially applies to the paleoclimate of Plio-Pleistocene glacial cycles, which played an important role in the evolution of many extant organisms (Hewitt, 2011). While there is an apparent impact of glacial cycles on Northern Hemisphere biota, there are contrasting hypotheses if and how glacial cycles influenced biota of (sub-) tropical regions, especially the African savannah biome. Beside this there is good evidence that a large portion of hybrid zones are the result of climatic changes leading to interspecific gene flow in secondary contact zones (Hewitt, 2011), which was also found in numerous African savannah mammals including primates. The role of hybridization in the evolution of animals is still regarded as less important compared to other more common evolutionary mechanisms. However, the more studies are conducted the more cases of hybridization become obvious.

In my thesis I will use African green monkeys (*Chlorocebus*) as a model to analyse major evolutionary trends in African savannah mammals during the Plio-Pleistocene and to contribute to the understanding of the impact of hybridization on the evolution of primates and animals in general.

In section 1.1 of this general introduction I will give an overview of different hypotheses on the African paleoclimate and its influence on the evolution of species with a focus on African savannahs, where African green monkeys occur. Section 1.2 addresses the growing interest in the study of hybridization and potential evolutionary outcomes of hybridization in animals, including primates. Following an overview of appropriate genetic markers to examine phylogeography and hybridization (section 1.3), I will highlight why African green monkeys represent an excellent model to study major evolutionary trends in African savannah mammals and the role of hybridization in the evolution of animals (section 1.4). Finally, I will describe major aims and approaches of my thesis in section 1.5.

1.1 Influences of Plio-Pleistocene climatic changes on speciation

Within the last decades analyses of different paleontological and geomorphological data sets revealed essential information to reconstruct the paleoclimate of the Pliocene (5.332-2.588 million years ago [mya]), Pleistocene (2.588-0.012 mya) and Holocene (0.012 mya - present), which offers an essential basis to study its impact on evolutionary processes (deMencoal, 1995; Nichol, 1999; deMenocal, 2004; Hewitt, 2004; Trauth et al., 2005; Andersen et al., 2006). Initially, the Early Pliocene was marked by a subtle warm and humid trend leading to expanded rain forests and extensive species richness on the African continent in the Late Pliocene (~3.48 mya; Zachos et al., 2001; Plana, 2004). This humid phase was replaced by an increasing drier climate around 3.2 mya, most probably reflecting the final separation of the Atlantic Ocean by the closure of the Isthmus of Panama, which was followed by the onset of high-latitude glacial cycles with characteristic alternating warm-humid and marked cold-arid periods (Haug et al., 2001; deMenocal, 2004). Henceforward, climatic fluctuations caused remarkable changes in global environments during the Plio-Pleistocene (Janis, 1993; deMenocal, 2004; Ehrich et al., 2007; Baker, 2008; Hewitt, 2011).

Plio-Pleistocene climate influences on evolutionary processes

It is well known that climate oscillations of glacial cycles also produced remarkable shifts in species distributions. Depending on the species' adaptation and tolerance, changes

from cold-arid to warm-humid periods and vice versa led to more or less intensive range retractions and extensions. In the Northern Hemisphere cold-arid glacial periods were marked by the expansion of ice sheets and glaciers, diminishing ranges of temperate adapted animals and plants to southern regions or low altitude areas. In contrast, warm-humid phases of interglacial periods allowed populations to expand and to recolonize deglaciated regions. Biological influences of glacials and interglacials has been intensively analysed in Northern Hemisphere and montane biomes (e.g., Brochmann et al., 2003; Schönswetter et al., 2005; Kotlík et al., 2006; Carstens & Richards, 2007; Herman & Searle, 2011; Hewitt, 2011; Pons et al., 2011). However, consequences of glacial climate oscillations on tropical and subtropical regions seem to be more complex and are discussed controversially. Assuming that climatic fluctuations of glacials and interglacials also affected tropical and subtropical regions, Haffer (1969) hypothesized that during cold-arid climatic periods Amazonian rain forests were isolated into forest fragments, which served forest-associated animals as refugia. This refuge hypothesis predicts that the isolation of populations into refugia induced genetic divergence and subsequent diversification of forest taxa. Subsequent studies testing this hypothesis found, however, less clear results and challenge the role of forest refugia in the evolution of the great biodiversity found in Amazonian rain forests (Pennington et al., 2000; Bush & Oliveira, 2006; Fouquet et al., 2012). Although less phylogenetic studies tested the forest refuge hypothesis in Africa, clearer results were found in African forest-associated taxa. Based on these findings forest refugia in West and Central Africa have been suggested (Grubb, 1982; Hamilton & Taylor, 1991; Anthony et al., 2007), but see Fjeldsa & Lovett (1997) for a different opinion).

A similar impact of high-latitude glacial cycles was proposed for the African savannah biome. Based on fossil as well as marine dust and pollen records increasing cold-arid climate conditions in the Pliocene coincided with expanding grasslands in Africa and consequently with the emergence of arid adapted faunal compositions between 2.9-2.4 mya, including the evolution of the hominin lineage (Cerling, 1992; DeMencoal, 1995; Vrba, 1999; deMenocal, 2004). Moreover phylogeographic analyses of diverse extant African savannah taxa indicate also influences of Plio-Pleistocene climate oscillations, comprising plants (Allal et al., 2011; Odee et al., 2012), carnivores

(Bertola et al., 2011), ungulates (Lorenzen et al., 2012), elephants (Nyakaana et al., 2002) and primates, like baboons (*Papio*; Zinner et al., 2009b, 2011b). In accordance with the forest refuge hypothesis, it is assumed that during warm-humid periods savannah areas retracted while rain forests expanded (Flagstad et al., 2001; Hassanin et al., 2007; Lorenzen et al., 2012; Smitz et al., 2013). Based on this assumption several savannah refugia were proposed in West, East, South and Southwest Africa (Lorenzen et al. 2012). Although the impact of humid periods on the savannah system by expanding forests seems plausible, the possibility remains that savannah areas were similarly affected by extreme dry climatic conditions. A southern extension of the Saharan desert up to one or two degrees of the equator is supported by fossil dunes (Nichol, 1999) and extreme dry conditions are also known from the southern East African Rift Valley (Scholz et al., 2007). Similar to significant humid periods, those extreme arid conditions might also have influenced the distribution of savannah mammals as it has been supposed for baboons (Zinner et al., 2011b).

Contrasting hypotheses on Plio-Pleistocene African climate

Although, there are some striking results, which support the hypothesis of the influence of glacial cycles on the global climate, there is evidence for a more complex pattern. Regional low-latitude climate changes superimposed the global climatic impact on environments at least in Northeast and Central Africa. Whereas deMenocal (1995, 2004) interpreted dust flux records as evidence for the coincidence of global cold-arid and high variability climates with marked faunal evolutionary steps in Africa, like the evolution of hominins and other savannah mammals, a statistical reanalysis of deMenocal's dust flux data by Trauth and colleagues (Trauth et al., 2009) showed a less consistent picture. They found a later increase in African climate variability and furthermore significant humid phases based on lake-level data in East Africa. Similarly, evidence for humid periods is also given by data on the sapropel formation in the Mediterranean Sea caused by increased freshwater discharge of the Nile River (Lourens et al., 2004) and a decrease of dust abundance in the East Mediterranean Sea (Larrasoana et al., 2003). However, it seems that alternating periods of extreme wetness and aridity superimposed the long-term drying trend in at least East Africa, where the hominin lineage evolved. Therefore Potts (1998, 2012) and Trauth et al. (2010) proposed that rapid shifts from humid to arid

conditions might have fundamentally influenced the evolution of hominins and also of other animals by triggering dispersal and speciation events.

While many studies discussed biogeographic and phylogenetic patterns of African savannah mammals with respect to high-latitude climatic influences of glacial cycles, the recent hypothesis of Trauth et al. (2010), which mainly considers the human evolution in East Africa, has not been tested in other savannah mammals yet. Therefore, the role of glacial climate oscillations in the evolution of African biomes remains questionable.

1.2 The role of hybridization in the evolution of animals

Hybridization in evolutionary research

According to Arnold (1997) "natural hybridization involves successful matings in nature between individuals from two populations, or groups of populations, which are distinguishable on the basis of one or more heritable characters" (p. 4). Advantages of Arnold's definition are the exclusion of any kind of experimental hybridization, and moreover it does not need the acceptance of one particular species concept (Arnold, 1997). Therefore I will adopt this definition here. In the beginnings, the phenomenon of hybridization was recognized as a dead-end in the evolution, due to the believed lower fitness and the high infertility rate of hybrid offspring. However, in plants hybridization and its notable role in evolutionary processes have long been accepted (reviewed in Arnold 1997, 2006). Contrastingly, interspecific gene flow was considered to be of little significance in diversification and adaptive evolution in animals until the early 20th century (Arnold, 1997; Arnold & Meyer, 2006). Based on molecular genetic analyses of cyprinid fishes Dowling and DeMarais (1993) discovered remarkable influence of hybridization in the evolution of vertebrates and indicated: "Botanists recognize the importance of introgressive hybridization in evolution. Our results […] indicate that zoologists must do the same" (Dowling & DeMarais, 1993, pp. 444-445). To date hybridization in animals is no longer recognized as a rare phenomenon, and it is known that hybridization can also have significant effects on evolutionary processes in animals (e.g., Mallet, 2005; Zinner et al., 2011a; Abbott et al., 2013).

In the past, our knowledge of hybridization was mainly based on morphological characters (often of museum specimens) or on observations of intermediate morphotypes in the field. Based on such approaches ancient hybridization, i.e. past hybridization events, cannot be detected. Moreover morphological evidence of hybridization is limited as hybrids might be indicative by only few genetic differences in colour genes (Mallet, 2005). The advances in molecular genetic methods in phylogenetic research, especially sequencing technologies, have contributed remarkably to the increasing knowledge on ancestral relationships among and between species and to the role of hybridization in the evolution of several vertebrates, including primates (Arnold, 1997; Zinner & Roos, 2010). Meanwhile, it is known that hybridization occurs on average in around 10% of animal and 25% of plant species. Thereby, hybridization occurs most frequently among closely related species, or lineages that diverged recently. Nevertheless, interspecific gene flow can often persist over some million years after the initial divergence of respective lineages (Mallet, 2005).

Outcomes of natural hybridization

In sense of a classic allopatric speciation model (Mayr, 1963), two populations or groups of populations diverge and evolve independently from each other as a response to geographic isolation. If previously isolated populations come into secondary contact, e.g. through recurrent environmental changes, and the time period was not long enough to accumulate effective differences and reproductive isolation mechanisms, then more or less successful gene exchange among lineages might still occur. Thereby interspecific gene flow can induce diverse evolutionary processes with different outcomes in respect of genetic diversity and the number of distinguishable lineages (Arnold, 1997; Arnold & Meyer, 2006; Zinner & Roos, 2010). If hybrids represent a low fitness or are less viable compared to parent lineages, favoured matings among conspecifics restrict interspecific gene flow (assortative mating), which in turn reinforces reproductive barriers and leads to the formation of a more or less stable and narrow hybrid zone (Mallet, 2005; Arnold, 2006). Alternatively, if hybrids do not show selective disadvantages in comparison to parent lineages, interspecific reproduction might lead to the formation of a new (hybrid) lineage, or to the amalgamation of previously diverged lineages resulting in the loss of diversity (Arnold & Meyer, 2006).

Whereas hybrid speciation is very common in plants and is often characterized by allopolyploidy (chromosome duplications in hybrids), hybrid speciation in animals seemed to be less common. Although, homoploid hybrid speciation became obvious in a growing number of animal taxa (Mallet, 2007). One example highlighting the capabilities of hybridization in the evolution of animals is the formation of a new fish lineage that evolved after two species of sculpins (*Cottus* sp.) came into secondary contact. In this example the new hybrid lineage was able to colonise habitats that were previously unsuitable for both parental species (Stemshorn et al., 2011). However, a more widespread form of hybridization in animals is introgressive hybridization. Introgression is the invasion of foreign genetic material into a genome through hybridization (Mallet, 2005). The transfer of certain genomic regions into foreign genomes occurs at different rates depending mainly on selection and drift. The result is a mosaic genome that shows portions of genomes from both hybridizing lineages (Arnold & Meyer, 2006). Furthermore, Haldane's rule (Haldane, 1922) predicts that hybrids of the heterogametic sex, e.g. the female in birds and the male in mammals, are preferentially sterile or inviable. Consequently, the transmission of maternal inherited mitochondrial DNA by introgression is less common in birds than in mammals (Orr, 1997; Mallet, 2005; Schilthuizen et al., 2011). The effect can be enhanced by sex-biased dispersal and by unequal effective population sizes of involved taxa, i.e. the resident taxon has a lower population size than the invading taxon (Zinner et al., 2011a). Introgression occurs preferably during colonization events, when previously isolated species or lineages get in secondary contact (Seehausen et al., 2008). Those secondary contact or hybrid zones are characterized by the genetic and ecological differentiation of hybridizing taxa, which also determines the stability or movement of the zone. If hybridization occurs symmetrically among lineages and environmental conditions remain stable the zone of contact will probably persist in the same geographic area for a longer period. Otherwise, asymmetrical hybridization, i.e. hybrids preferentially backcross with only one of the parental lineages, leads typically to the movement of the zone (Buggs, 2007). In extreme cases, hybrid zones can overrun the complete distribution of one of the parental species, which can cause the cytonuclear extinction of the invaded lineage, leaving "relict" extra-nuclear genomes behind, for example the mitochondrial genome in mammals, (Buggs, 2007; Zinner & Roos, 2010). Depending on the dispersing sex as well as on the parental

lineage with which backcrossing preferentially occurs (invader vs. resident) possible outcomes of unidirectional introgression in mammals are mitochondrial or Y-chromosomal capture (backcrossing with resident lineage) or nuclear swamping (backcrossing with invading lineage) (Zinner et al., 2011a).

Introgressive hybridization may represent a challenge for phylogeneticists, since it can produce reticulated phylogenies and discordant patterns depending on the markers that are used to elucidate genetic relationships. Due to different introgression patterns the analysis of different markers, especially those which are inherited by only one sex, might result in para- and polyphylies or in conflicting phylogenies with discordances between genetic loci, morphology and geographic pattern (Funk & Omland, 2003).

The role of hybridization in the evolution of primates

Hybridization producing reticulate and discordant phylogenies is evident in nearly all major radiations of primates, including strepsirrhines, tarsiers, New World monkeys, Old World monkeys, apes and even humans. The application of molecular methods revealed several instances of ancient hybridization in primates, which were, in contrast to contemporary hybridization, not detectable in the field or by using morphological data (Zinner et al., 2011a). Currently, the occurrence of hybridization is known in more than 10% of the recognized primate species (Arnold & Meyer, 2006; Zinner & Roos, 2010; Zinner et al., 2011a). Introgressive hybridization is more common than bidirectional hybridization and occurred not only between species but also among different genera, e.g. between *Papio* and *Rungwecebus* (Zinner et al., 2009a; Roberts et al., 2010), between *Cercopithecus* and *Chlorocebus* (de Jong & Butynski, 2010) or between *Trachypithecus* and *Semnopithecus* (Roos et al., 2011). However, also bidirectional hybridization can occur in primates as indicated by the mosaic genome of *Macaca arctoides* that resulted from hybridization among a proto-*M. fascicularis* group and an early *M. sinica* group population (Tosi et al., 2003; Li et al., 2009). Hybridization has been intensively studied in baboons (*Papio*), which are widely distributed in African savannahs. Those studies indicate contemporary hybridization in current contact zones (Tung et al., 2008; Jolly et al., 2011; Charpentier et al., 2012) as well as ancient introgressive hybridization among most species assuming several instances of population reduction and expansion in response to changing climates and environments

during the Plio-Pleistocene (Newman et al., 2004; Zinner et al., 2009b; Keller et al., 2010). Since most baboon species show a male biased dispersal pattern, discordances between morphology and mitochondrial DNA were explained by male-mediated introgression and nuclear swamping (Keller et al., 2010). Thereby, in some cases discordances between morphology and mitochondrial DNA revealed the complete extinction of historical populations or taxa, whose mitochondrial genomes remained only as traces in extant taxa, e.g. in olive or gray-footed chacma baboons (Zinner et al., 2009b). Evidence of introgressive hybridization was even found in the hominin lineage indicated by mosaic genomes. The analysis of the Neanderthal genome in comparison to modern human genomes revealed that 1-4% of the non-African modern human genome was of Neanderthal ancestry (Green et al., 2010). Moreover, comparisons of modern human and Denisovan genomes elucidated that 6% of the present-day Papuan genome derives from Denisovans (Meyer et al., 2012). Even more interesting, another comparison found introgression from Denisovan archaic alleles that are at present involved in the immune system of modern Eurasian humans (Abi-Rached et al., 2011). This example of adaptive introgression accentuates the potential importance of hybridization in the evolution of primates and other animals.

Why study hybridization?

Charles Darwin already said, “Those forms which possess in some considerable degree the character of species, but which are so closely similar to some other forms, or are so closely linked to them by intermediate gradations, that naturalists do not like to rank them as distinct species, are in several respects the most important to us.” (Darwin, 1859, p. 47). Even though successful hybridization still remains a minor evolutionary factor compared to other common evolutionary forces, several examples accentuate the capability of hybridization to produce genetic diversity as well as to serve as a basis for adaptive evolution. Above examples show that evidence of hybridization increases with increasing molecular genetic studies of diverse taxa. Thus, phylogenetic studies testing further hybridization events in additional taxa might help us to get a more complete picture of the degree and role of hybridization in the evolution of animals in general, and of primates including us humans.

1.3 Molecular markers and approaches to study phylogeography and hybridization

Mitochondrial DNA is highly suited to measure geographic variations and genetic relationships of animals and has been successfully used in a diverse array of studies investigating phylogeny, phylogeographic patterns, and population demography within and among species (Avise, 1998). Advantages of using mitochondrial DNA to study phylogeography of closely related species are based on the fast evolutionary mode compared to nuclear DNA, its haploid nature and the predominant uniparental mode of maternal inheritance without recombination, which reduces the effective population size, and in turn, the time of fixation (Avise, 2004, 2009).

However, in case of (introgressive) hybridization the analysis of solely mitochondrial DNA is inappropriate due to its predominant maternal inheritance. Therefore certain hybridization events, like male-mediated introgression, would not be identified. Only the analysis of different unlinked genetic loci or marker systems enables the detection of ancient and on-going hybridization among lineages and to rule out sorting failures, i.e. homoplasy, incomplete lineage sorting and hemiplasy, which produce similar genetic patterns as hybridization (Avise & Robinson, 2008; Kubatko, 2009). Consequently, a combination of loci from mitochondrial, paternal inherited Y-chromosomal, and autosomal DNA should be ideally analysed. Since morphological characters are more likely to be consistent with nuclear than with mitochondrial DNA (Zinner et al., 2009b), morphological data can also be used to infer information on the nuclear genome.

To characterize general evolutionary trends that occurred in certain regions or biomes comparative phylogeographic approaches has been proven to be a useful tool. Concordant patterns of certain biogeographic clades of co-distributed taxa or the temporal coincidence of population divergence and expansions are indications of possible ancient refugia, dispersal corridors and suture zones as well as the evidence of time periods, in which major changes occurred in respective biomes (Bermingham & Moritz, 1998; Bernatchez & Wilson, 1998; Taberlet et al., 1998; Moritz et al., 2009; Morgan et al., 2011; Fouquet et al., 2012). Therefore, comparative phylogeographic studies of multiple co-distributed species enable the detection of salient historic events like pronounced environmental changes in response to climatic shifts (Avise, 2009).

1.4 African green monkeys (*Chlorocebus*) as a model

African green monkeys of the genus *Chlorocebus* are widely distributed in sub-Saharan Africa and their current range almost reflects the present extension of African savannahs (Hill, 1966; Lernould, 1988; Kingdon, 1997). Little is known on their genetic diversity, which is grounded on the analysis of only few samples with uncertain origins or with focus on a small region in Ethiopia (van der Kuyl et al., 1995; van der Kuyl, 1996; Shimada & Shotake, 1997; Shimada, 2000; Shimada et al., 2002; Wertheim & Worobey, 2007). However, based on morphological data the genus represents a diverse group and up to 25 taxa were described (Dandelot, 1959; Hill, 1966; Napier, 1981). Although most of them are currently regarded as synonyms, the intra-generic taxonomy is still disputed and depending on author the genus comprises four to six species with several subspecies (Dandelot, 1971; Groves, 2001, 2005), or alternatively, *Chlorocebus aethiops* is treated as one polytypic species with six subspecies (Grubb et al., 2003; Elton et al., 2010). In my thesis I will follow the recent taxonomy of Groves (Groves, 2001, 2005) and Anandam et al. (in press), who recognize six species (*Chlorocebus aethiops*, *C. tantalus*, *C. cynosuros*, *C. djamdjamensis*, *C. pygerythrus*, and *C. sabaeus*) and eight subspecies (*C. tantalus tantalus*, *C. t. budgetti*, *C. t. marrensis*, *C. pygerythrus pygerythrus*, *C. p. hilgerti*, *C. p. excubitor*, *C. p. nesiotes*, *C. p. rufoviridis*).

Since African green monkeys play an important role in biomedical and especially in virus research (Müller & Barré-Sinoussi, 2003; Switzer et al., 2005), mitochondrial genomes of four of the currently recognized species have been analysed for a co-evolutionary study on simian immunodeficiency virus (SIV) and its specific hosts (Wertheim & Worobey, 2007). Although results of this study suggest an origin of the genus in the Early Pleistocene and rejected co-evolution between host and virus species, their conclusions should be treated carefully, since the analysis of samples from only four species with uncertain origin most probably does not provide a reliable phylogeny of this morphological diverse genus. Moreover, based on morphological, fossil and genetic data currently three controversial phylogeographic scenarios exist with possible origins of the genus in East, South and West Africa (Figure 1.1; Schwarz, 1926; Hill, 1966; Kingdon, 1984; Elton, 2007).

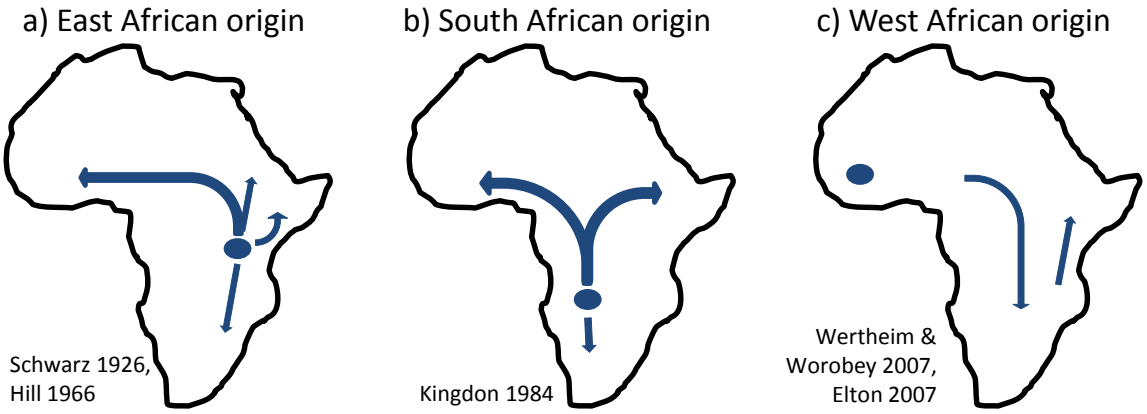

Figure 1.1 Different phylogeographic scenarios with origins of the genus in a) East, b) South, and c) West Africa based on morphological (a and b) or on fossil and genetic data (c). Ovals represent presumed origins of the genus.

If Plio-Pleistocene climatic changes have influenced the evolution of African green monkeys in a similar way as the evolution of other savannah mammals, like baboons, we expect that recurrent range retraction and expansion also favoured the formation of secondary contact zones and the occurrence of hybridization. Currently, there are no genetic data supporting this hypothesis, but based on information from morphology, contemporary hybridization has been reported from contact zones among most African green monkey species in East Africa (Napier, 1981; Kingdon, 1997; Mekonnen et al., 2012). This also includes the endemic Bale monkey *C. djamdjamensis*, which occurs only in a small area in the highlands of Southeast Ethiopia (Kingdon, 1997; Mekonnen et al., 2010a, 2010b, 2012). On-going hybridization between this species and neighbouring lowland forms (*C. aethiops* and *C. pygerythrus*) is supposed to represent a possible risk to the survival of *C. djamdjamensis* (Kingdon, 1997; Mekonnen et al., 2012). In contrast, less evidence of hybridization is found in other contact zones. In West Africa the species *C. sabaeus* and *C. tantalus* are apparently separated by the White Volta River in Ghana (Booth, 1956, 1958). However, farther to the north in Burkina Faso, where confluents of the Volta River do not represent geographic barriers, hybridization is highly probable (Lernould, 1988). Although these data indicate that hybridization occurs within the genus to a certain extent, they do not provide information on historical hybridization events, nor on the degree and direction of gene flow among species.

While molecular genetic analyses of diverse savannah mammals in Africa including primates and ungulates indicated an influence of Plio-Pleistocene climatic changes on their evolution (e.g., Arctander et al., 1999; Muwanika et al., 2003; Zinner et al., 2009b, 2011b; Lorenzen et al., 2010, 2012), only a limited number of studies estimated divergence ages within species or genera. Furthermore the application of different calibration methods to estimate divergence time hampers a taxon-wide comparison (Lorenzen et al., 2012). Whereas the range of African green monkeys covers wide areas of African savannahs, distributions of many other savannah mammals are restricted to certain areas (Kingdon, 1997), which would provide only incomplete information on historical changes in the savannah biome. Bearing in mind the uncertainties in the African Plio-Pleistocene climate and the question of how African savannah mammals were affected by respective environmental shifts, a comparative phylogeographic approach of African green monkeys and other widely distributed savannah mammals is a promising approach to get deeper insights into evolutionary processes of African savannah mammals.

1.5 Aims and approaches

The aim of my thesis is to analyse the phylogeography of African green monkeys and the degree and pattern of their interspecific gene flow to contribute to a better understanding of evolutionary trends in African savannah mammals. Therefore I aim to analyse the evolutionary history of African green monkeys by studying (1) the genetic diversity and biogeography of the currently six acknowledged species, (2) the degree and pattern of intra-generic hybridization, and (3) their temporal and spatial phylogeographic patterns, and finally, (4) I intend to compare obtained results on African green monkeys with phylogeographic patterns of three other widely distributed savannah mammals to examine general evolutionary trends in African savannahs.

For these purposes I collected faecal samples and morphological information from five of the six African green monkey species in three African countries with a special emphasis on two contact zones in West Africa (Ghana and Burkina Faso) and in Ethiopia. As these data did not provide efficient information on the genus' wide distribution I completed this data set with museum samples from two German and one Kenyan

museum and with hair and faecal samples that I obtained from previous projects of the German Primate Center. I used the complete data set, representing all six species and large proportions of the genus' range, to investigate the diversity and biogeography of African green monkeys and to detect potential evidence for hybridization in contact zones by analysing the complete mitochondrial cytochrome *b* gene in comparison to morphology and geographic distribution (Chapter 2). To test findings on potential new (mitochondrial) lineages of the first study, and to reveal patterns of introgressive hybridization I further analysed two Y-chromosomal markers with focus on the West African contact zone, where hybridization was previously assumed (Chapter 3). Finally, I applied a comparative phylogeographic approach to get a more complete picture of African savannah mammal evolution using my data on African green monkeys and already published mitochondrial sequence data of baboons (*Papio* spp.), hartebeests (*Alcelaphus* spp.) and warthogs (*Phacochoerus africanus*) (Chapter 4). In Chapter 5 I summarize and discuss findings of these studies with respect to previous results of African green monkeys and to the role of introgressive hybridization in the evolution of animals and its potential influence on genetic and species diversity, and further, I address major trends in the evolution of the African savannah biome.

CHAPTER 2

Mitochondrial Diversity and Distribution of African Green Monkeys (*Chlorocebus* Gray, 1870)

Tanja Haus[1,2], Emmanuel Akom[4], Bernard Agwanda[5], Michael Hofreiter[6], Christian Roos[2,7], and Dietmar Zinner[1]

1 Cognitive Ethology Laboratory, German Primate Center, Leibniz Institute for Primate Research, Goettingen, Germany

2 Primate Genetics Laboratory, German Primate Center, Leibniz Institute for Primate Research, Goettingen, Germany

3 Centre of Biodiversity and Sustainable Land Use, University of Göttingen, Göttingen, Germany

4 College of Agriculture and Natural Resources, Kwame Nkrumah University of Science and Technology, Kumasi, Ghana

5 Mammalogy Section, National Museums of Kenya, Nairobi, Kenya

6 Department of Biology, University of York, York, UK

7 Gene Bank of Primates, German Primate Center, Leibniz Institute for Primate Research, Goettingen, Germany

CR and DZ contributed equally

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Abstract

African green monkeys (*Chlorocebus*) represent a widely distributed and morphologically diverse primate genus in sub-Saharan Africa. Little attention has been paid to their genetic diversity and phylogeny. Based on morphological data six species are currently recognized, but their taxonomy remains disputed. Here we aim to characterize the mitochondrial (mt) DNA diversity, biogeography and phylogeny of African green monkeys. We analyzed the complete mitochondrial cytochrome *b* gene of 126 samples using feces from wild individuals and material from zoo and museum specimens with clear geographical provenance, including several type specimens. We found evidence for nine major mtDNA clades which reflect geographic distributions rather than taxa, implying that the mtDNA diversity of African green monkeys does not conform to existing taxonomic classifications. Phylogenetic relationships among clades could not be resolved suggesting a rapid early divergence of lineages. Several discordances between mtDNA and phenotype indicate that hybridization may have occurred in contact zones among species, including the threatened Bale monkey (*Chlorocebus djamdjamensis*). Our results provide both valuable data on African green monkeys' genetic diversity and evolution and a basis for further molecular studies on this genus.

Key words: cytochrome *b* gene, phylogeny, hybridization, introgression, African savanna

INTRODUCTION

African green monkeys of the genus *Chlorocebus* occur in savanna habitats across sub-Saharan Africa (Fig. 2.1; Lernould, 1988; Kingdon, 1997). Previously, African green monkeys have been subsumed into the *aethiops* group of the genus *Cercopithecus* (Schwarz, 1926; Hill, 1966; Dandelot, 1971; Napier, 1981; Grubb et al., 2003), but based on recent morphological and genetic studies they are now separated from *Cercopithecus* and placed within the genus *Chlorocebus* as sister taxon to the other ground dwelling members (*Erythrocebus, Allochrocebus*) of the Cercopithecini (Groves, 2001, 2005; Tosi et al., 2002; Xing et al., 2007; Mekonnen et al., 2010a, 2010b; Perelman et al., 2011; but see Grubb et al. (2003) for a different opinion). Due to their wide distribution and phenotypic diversity 22 taxa have been described with most of them now being recognized as synonyms (Schwarz, 1926; Hill, 1966; Dandelot, 1971; Napier, 1981; Groves, 2001). However, their taxonomy is still disputed and some researchers consider *Chlorocebus aethiops* as one polytypic species comprising five or six subspecies (Kingdon, 1997; Grubb et al., 2003; Elton et al., 2010), whereas Dandelot (1971) preferred a classification with four species and several subspecies. Here we follow the taxonomy of Groves (2001, 2005) as his classification combines the most recent findings on genetics, morphology, and ecology on generic as well as on species and subspecies level. He recognizes six species which is also followed by the IUCN red list of threatened species (IUCN, 2012); four monotypic species: *C. aethiops* (grivet), *C. djamdjamensis* (Bale monkey), *C. sabaeus* (green monkey) and *C. cynosuros* (malbrouck monkey), and two polytypic species: *C. tantalus* (tantalus monkey) with subspecies *C. tantalus budgetti*, *C. t. marrensis* and *C. t. tantalus*, and *C. pygerythrus* (vervet) with subspecies *C. pygerythrus hilgerti*, *C. p. excubitor*, *C. p. nesiotes*, *C. p. rufoviridis* and *C. p. pygerythrus*.

Whereas most species inhabit wide geographic ranges and are listed as “Least Concern” in the IUCN red list of threatened species (Butynski, 2008; Kingdon and Butynski, 2008; Kingdon and Gippoliti, 2008a,b; Kingdon et al., 2008), *C. djamdjamensis* is endemic to the highlands of South Ethiopia and is classified as “Vulnerable” (Butynski et al., 2008). In addition to ongoing habitat disturbance, Kingdon (1997) assumed that *C. djamdjamensis* is additionally threatened by hybridization with the lowland forms

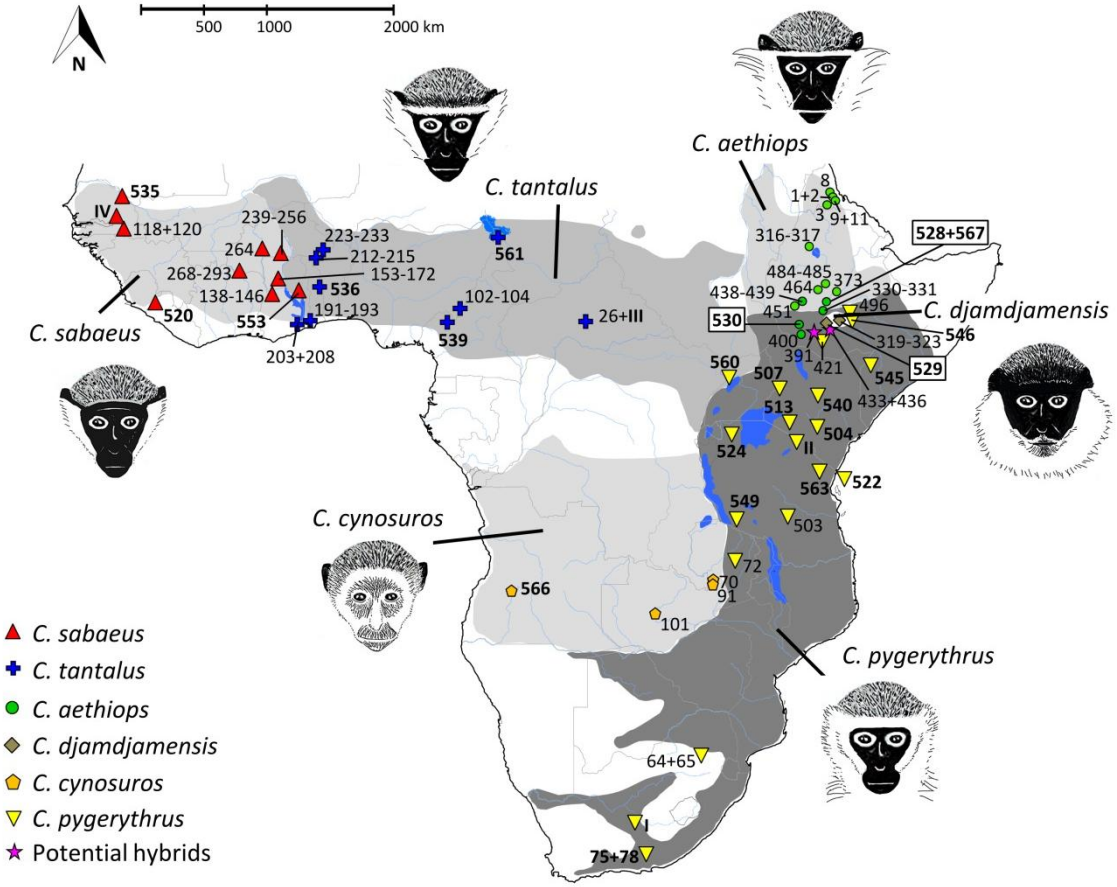

Fig. 2.1 Distribution of African green monkeys (*Chlorocebus*) and collection sites of fecal and museum (bold) samples. Species distributions are shaded and modified from Lernould (1988) and Kingdon (1997). Colored symbols indicate phenotypes determined. Numbers correspond to IDs in Fig. 2.2 and Table S2.I. IDs of type specimens are boxed. Schematic drawings depicting main differences in facial characters are redrawn from Hill (1966).

C. aethiops and *C. pygerythrus*. Hybridization seems to be not uncommon within *Chlorocebus* as it has been reported from most species contact zones in East Africa, and even inter-generic hybridization with *Cercopithecus mitis* has been observed (Napier, 1981; Kingdon, 1997; de Jong & Butynski, 2010; Mekonnen et al., 2012). However, with respect to *C. djamdjamensis*, both its taxonomic status and the potential threat by hybridization remain unclear without genetic analyses.

Whatever taxonomic classification is applied to the members of *Chlorocebus*, the phylogenetic relationships among the different taxa are unresolved and a comprehensive phylogenetic analysis has yet to be done (Groves, 2001; Grubb et al.,

2003). Even though African green monkeys are used as model organism in biomedical research, e.g. in Simian Immunodeficiency Virus (SIV) research (Switzer et al., 2005; Wertheim & Worobey, 2007), only few intra-generic genetic studies have been conducted, either focusing on a small region in Ethiopia (Shimada, 2000; Shimada et al., 2002) or relying on only small and taxonomically incomplete data sets (van der Kuyl et al., 1995; van der Kuyl, 1996). Based on this data the origin of green monkeys from the Caribbean islands (Barbados, St. Kitts, and Nevis), which are widely used in biomedical research, was assumed to be in Senegal or Gambia (van der Kuyl, 1996). Although complete mitochondrial genomes were analyzed in a study on co-evolutionary processes between African green monkeys and their host-specific SIVs (Wertheim & Worobey, 2007), a total of just six individuals of four taxa representing only a small part of the genus' geographical range were included in this study.

Given the conflicting taxonomic classifications and the lack of genetic analyses covering all major *Chlorocebus* taxa and the complete geographical range of the genus we attempt to clarify the genetic diversity of African green monkeys using a comprehensive data set representing all species. In the present study, we analyzed the complete mitochondrial cytochrome *b* (cyt *b*) gene from 126 African green monkey samples with the aim to evaluate the mitochondrial diversity within the genus, to delineate geographic ranges of taxa and to elucidate their phylogenetic relationships.

METHODS

Sample collection

We collected 91 fecal samples of wild African green monkeys originating from 32 sites in Senegal, Ghana, Burkina Faso, Nigeria, Ethiopia, Zambia, Tanzania, and the Republic of South Africa (RSA) sampled between 2005 and 2010 (Fig. 2.1, Table S2.I). Samples were kept for at least 24h in >90% ethanol and, after drying, stored on silica beads (Nsubuga et al., 2004). Only few samples from Nigeria and Zambia were stored directly on silica (dry samples) or only in ethanol (fresh samples). We determined geographic coordinates of sample localities using GPS (Table S2.I). We further included seven hair samples from zoos and 24 museum samples (skin, dried soft tissue or teeth, Table S2.I), including

samples of holotypes of *djamdjamensis* (sample ID 529), *ellenbecki* (sample ID 528), and *matschiei* (sample ID 530), and a paratype of *ellenbecki* (sample ID 567, Fig. 2.1). We used only museum samples with clear provenance. As there is no detailed information on the origin of the hair sample from the Central African Republic (CAR, ID 26) we depicted the locality in the center of the country (Fig. 2.1). For museum samples we used approximate coordinates of sampling sites based on voucher localities. We complemented our sample set with already published sequences of four individuals from Senegal, Tanzania/Kenya, CAR and RSA (Wertheim & Worobey, 2007). As the exact origins of these individuals are unknown, we depicted approximate sample localities in the map according to van der Kuyl et al. (1996) (I-IV in Fig. 2.1).

For the determination of species (according to the taxonomy of Groves (2001)) in the field and of museum specimens we applied chief distinguishing phenotypic characters by direct observation (Hill, 1966; Napier, 1981; Groves, 2001). For facial pattern we recorded in particular information on color and structure of whiskers and the white frontal band as well as on the presence of the white mustache (Fig. 2.1). Further we used the presence of the paracaudal white tuft and the subcaudal red patch, and information on the color of extremities and tail tips. As we do not have phenotypic information on analyzed specimens from Zambia, CAR and GenBank we assigned them to species according to their geographical provenance. In total our data set comprises 126 samples from 59 sites representing all six proposed *Chlorocebus* species (Fig. 2.1).

All research in this project complied with protocols approved by the German Primate Center in Germany, Ethiopian Wildlife and Conservation Authority (EWCA) in Ethiopia, the Centre National de la Recherche Scientifique et Technologique (CNRST) in Burkina Faso, the Forestry Commission (FC) of Ghana, and the National Museums of Kenya (NMK) in Kenya, and adhered to the legal requirements of the countries in which the research was conducted. The study was carried out in compliance with respective animal care regulations and the principles of the American Society of Primatologists for the ethical treatment of non-human primates.

Extraction, amplification and sequencing of DNA

Extraction of total genomic DNA from fecal samples was performed with the QIAamp DNA Stool Mini Kit (Qiagen, Germany) following standard protocols with only minor changes. Samples were incubated in ASL buffer overnight and DNA was eluted in 210 µl water (high-performance liquid chromatography (HPLC) grade) instead of AE buffer. The extracts were stored in 50 µl aliquots at -20°C for up to 24 months before further processing. For analysis of hair samples from zoos roots of 3-5 hairs were directly added to the polymerase chain reaction (PCR) mix without prior DNA extraction (Fontanesi et al., 2007; Roos et al., 2008). For the extraction of museum samples (teeth, pelt/skin, dried soft tissue) we used a Guanidinium thiocyanate (GuSCN) buffer (5M GuSCN, 25 mM NaCl, 50 mM Tris, 20 mM EDTA, 1% Tween 20, 1% beta-mercaptoethanol) modified from Rohland et al. (Rohland et al., 2004). Samples were incubated for about 24h in 1 ml extraction buffer per 50 mg sample under constant agitation at room temperature in the dark. We purified DNA with a combination of a batch-based silica and a column-based method according to Rohland & Hofreiter (2007) and Rohland et al. (2010), and eluted the DNA in 50 µl TE buffer. To avoid contamination with modern DNA, extractions of museum samples were conducted in a laboratory dedicated to ancient DNA analysis at the University of York. To monitor for possible cross-sample contamination we performed one to three blank extractions (without sample) per extraction depending on the number of samples processed.

Since in mammals with female philopatry mtDNA is known to conserve geographical pattern better than nuclear DNA (Avise, 2009), we analyzed the mitochondrial cyt *b* gene, which has been successfully used to resolve phylogenetic relationships in several mammals (Castresana, 2001; Agnarsson & May-Collado, 2008; Roos et al., 2008; Thinh et al., 2010; Tobe et al., 2010). We amplified the complete cyt *b* (1,140bp) gene via two or four (fecal samples), or even six (museum samples) overlapping fragments (Table S2.II), because most of our samples are expected to yield only degraded DNA. For hair samples only we used a nested PCR approach with external primers first, and subsequently, with primers amplifying two overlapping fragments in separate PCR reactions. We used 1 U BiothermTaq 5000 (Genecraft, Germany) for hair and fecal samples in a 30 µl PCR mix (1x reaction buffer, 0.16 mM for each dNTP,

0.33 µM for each primer, and 0.6 mg/ml BSA), with the following thermo cycler conditions: 94°C for 2 min, followed by 40 cycles of 94°C for 1 min, 62°C for 1 min, 72°C for 1 min, and 72°C for 5 min. For the amplification of museum samples we used 3 U AmpliTaq Gold 360 (Applied Biosystems, Germany) in a 20 µl mix (1x reaction buffer, 2 mM $MgCl_2$, 0.25 mM for each dNTP, 0.75 µM for each primer, and 0.1 mg/ml BSA) and the following PCR conditions: 94°C for 10 min, followed by 60 cycles of 94°C for 30 s 62°C for 45 s, 72°C 45 s, and 72°C for 5 min. To test for reliability of sequences generated from museum samples, we randomly replicated at least two of the six cyt *b* fragments for each sample. For 11 museum samples, for which we found putative nuclear insertions of mitochondrial sequences (NUMTs) in one or two of the six fragments, we amplified longer fragments (up to 555 bp). PCR reactions were conducted with one or two PCR blanks (HPLC-purified water) in addition to the extraction blanks depending on the number of samples processed. We ran all PCR products on 1-2% agarose gels and, after excision, purified PCR products with the Qiagen Gel Extraction Kit (Qiagen, Germany). Subsequently, sequences were run on an ABI 3130*xL* sequencer using the BigDye Terminator Cycle Sequencing Kit (Applied Biosystems, Germany) and respective forward and reverse primers. All sequences were deposited in GenBank (for GenBank accession numbers see Table S2.I).

Statistical analyses

We assembled and aligned sequences with the program Geneious Pro 5.0.4 (Drummond et al., 2011) and corrected them by eye. To check for the presence of NUMTs, simple neighbor-joining trees for gene fragments were reconstructed in Mega 5.0 (Tamura et al., 2011) and branch lengths and depicted relationships were visually checked to be similar. Furthermore, all sequences were translated into amino acid sequences to detect unexpected stop codons.

We applied maximum-likelihood (ML) and Bayesian approaches for phylogenetic tree reconstructions using the programs Garli 2.0 (Zwickl, 2006) and MrBayes 3.1.2 (Huelsenbeck et al., 2001; Ronquist & Huelsenbeck, 2003). For tree reconstructions we used only unique sequences; therefore, the final alignment included 68 haplotypes of African green monkeys and one ortholog of *Erythrocebus patas* used as outgroup. For both reconstructions, the appropriate model of nucleotide substitution (TrN + G) was

chosen according to the Bayesian Information Criterion (BIC) as implemented in jModeltest 0.1 (Guindon & Gascuel, 2003; Posada, 2008). For the ML analysis support of internal nodes was assessed by 500 bootstrap replications in four independent runs. All other settings were left at their default value. A 50% majority-rule consensus tree was calculated with Paup* 4b10 (Swofford, 2003). For Bayesian reconstructions we applied 10 million generations with tree and parameter sampling every 10,000 generations. We checked the output of MrBayes for the adequacy of effective sample size (ESS) values and discarded the first 25% of sampled trees and parameters from the beginning of the chain as burn-in.

We used Network 4.610 (Bandelt et al., 1999) to additionally explore biogeographic patterns and to compare patterns of phenotype and mtDNA. We calculated a median-joining network based on cyt *b* sequences of the complete data set including all 126 samples. Results were displayed and edited using the Network Publisher software. To compare intra- and interspecific distances of obtained mtDNA clades or lineages we used the software Mega 5.0 (Tamura et al., 2011). We calculated the number of substitutions per site between sequences with the Tamura-Nei model and a gamma distribution.

RESULTS

We successfully amplified and sequenced the complete cyt *b* gene from 122 samples. Together with four sequences from GenBank the data set comprised 126 African green monkey sequences. Among them, we detected 68 unique haplotypes, which are characterized by a total of 329 variable sites of which 226 are parsimony-informative.

Based on directly observed phenotypic characters we clearly assigned samples from all regions to one of the six recognized *Chlorocebus* species (Fig. 2.1), except of some samples from Ethiopia (pink stars in Fig. 2.1), where we found phenotypes showing mixed characters of *C. aethiops* and *C. djamdjamensis* (sample IDs 433 and 436) or phenotypes of both *C. aethiops* and *C. pygerythrus* within one group (sample ID 391).

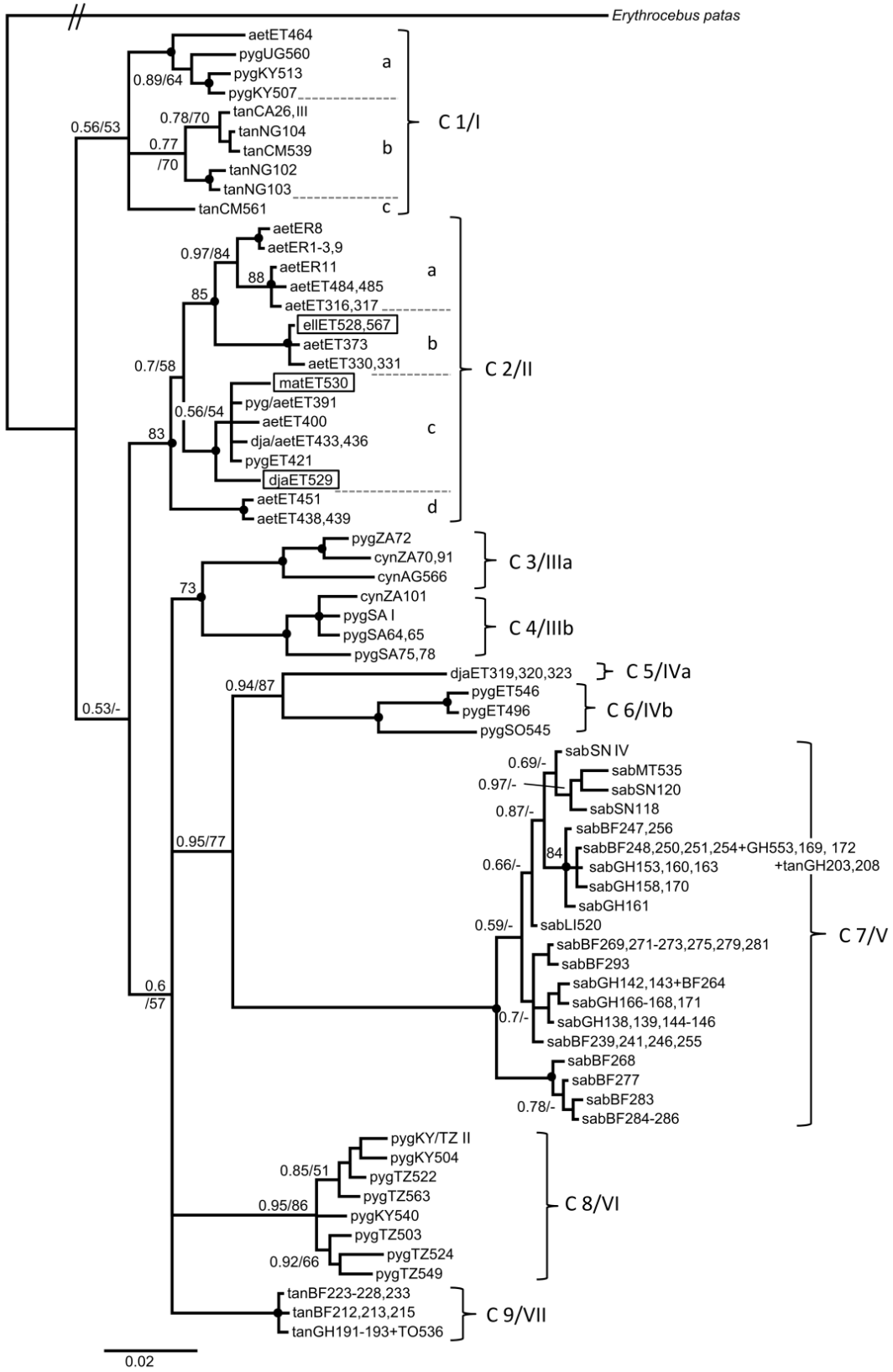

Fig. 2.2 Bayesian phylogram with posterior probabilities and ML bootstrap support values based on the complete cyt *b* gene. C1/I-C9/VII indicate main mtDNA clades. Bootstrap support values of >90% and posterior probabilities of >0.98 are presented as black dots; values below are given at respective nodes. Type specimens are boxed.

ML and Bayesian tree reconstructions resulted in nearly identical tree topologies showing seven or nine major mtDNA clades or lineages (C1/I-C9/VII, hereafter clades, Fig. 2.2). Monophyly of several clades is not strongly supported and these clades might be further divided into several subclades (C1/I a-c, C2/II a-d, C IIIa-b, and C IVa-b, respectively, Fig. 2.2). We additionally examined genetic differences within and among clades, which show profound overlap of genetic distances in C III and C IV when determining only seven clades supporting a division into nine major mtDNA clades (Fig. S2.1). In the median-joining network the same nine clades became apparent revealing good correspondence to geographic regions, except for the *C. aethiops* sample from Ethiopia, which falls together with samples from Nigeria, Cameroon, CAR, Uganda, and Kenya into C1 (Fig. 2.3). Phylogenetic relationships among clades remained largely unresolved due to low statistical support of both ML and Bayesian approaches (Fig. 2.2). The nine major clades comprise the following species and type specimens as delineated by phenotypes and geographic regions: C1 - *C. tantalus* from Nigeria and CAR, *C. aethiops* from Woliso in Ethiopia, and *C. pygerythrus* from Uganda and Kenya; C2 - *C. aethiops* from Ethiopia and Eritrea, the type specimens of *ellenbecki* and *matschiei*, *C. djamdjamensis* from Bubbe Kersa and Gossa, the holotype of *djamdjamensis* from Abera, and *C. pygerythrus* from Yabello in South Ethiopia; C3 - *C. cynosuros* from Angola and Northwest Zambia, and *C. pygerythrus* from Northeast Zambia; C4 - *C. pygerythrus* from South Africa, and *C. cynosuros* from South Zambia; C5 - *C. djamdjamensis* from the Bale Mountains National Park (NP) in Ethiopia; C6 - *C. pygerythrus* east of the Bale Mountains in Ethiopia and Somalia; C7 - *C. sabaeus* from West Africa west of the Volta and Oti River and *C. tantalus* from Shai Hills Resource Reserve (West of the Volta River); C8 - *C. pygerythrus* from Kenya and Tanzania; and C9 - *C. tantalus* from east of the Volta and the Oti River in Ghana, Burkina Faso and Togo (Figs. 2.2 and 2.3). With exception of *C. sabaeus* (C7) phenotypes of all species are found in more than one major clade due to discordance between phenotype and mtDNA (*C. tantalus*: C1, C7, C9; *C. aethiops*: C1, C2; *C. cynosuros*: C3, C4; *C. pygerythrus*: C1, C2, C3, C4, C6, C8; *C. djamdjamensis*: C2, C5) causing several instances of paraphyly within the genus *Chlorocebus* (Figs. 2.2 and 2.3).

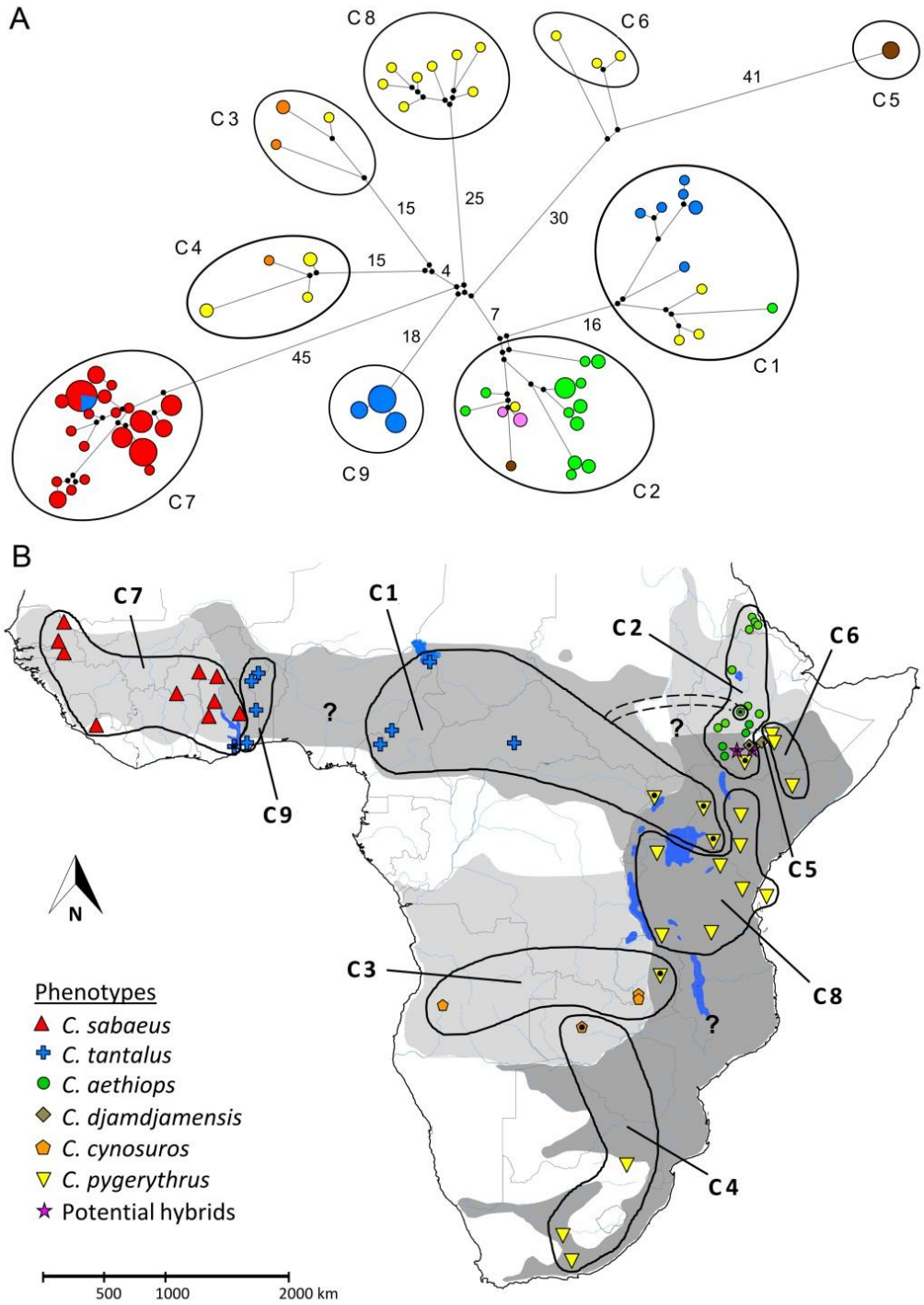

Fig. 2.3 (A) Median-joining network of mtDNA sequences with depicted clade affiliations (see Fig. 2.2). Sizes of circles indicate haplotype frequencies and colors represent different phenotypes. Black dots along branches represent median vectors and branch length is relative to the number of mutated positions. (B) Map showing geographic distribution of the mtDNA clades detected. Samples indicating discordance between mtDNA and observed phenotype are highlighted with black dots. Question marks indicate recommended regions for future studies.

DISCUSSION

Our results show that the mtDNA diversity does not conform to existing taxonomic classifications (Dandelot, 1959, 1971; Hill, 1966; Napier, 1981; Lernould, 1988; Kingdon, 1997; Groves, 2001, 2005), neither if we apply a six species classification nor a one superspecies classification. Furthermore, several discordances between phenotype and mtDNA, which are exclusively found in samples from regions close to contact zones among species (Fig. 2.3), point to possible hybridization. Hybridization and consequential discordance between mtDNA and nuclear DNA is a common pattern in cercopithecines (Detwiler et al., 2005; Zinner et al., 2009a, 2009b, 2011a; Keller et al., 2010). Since pheno- or morphotypes are more likely to be consistent with nuclear than with mtDNA phylogenies (Zinner et al., 2009b), we assume that introgressive hybridization is responsible for the discordances in our phylogeny of African green monkeys and that intra-generic gene flow is common among all *Chlorocebus* species. Thereby introgression would not vanish if a subspecies taxonomy is applied. The exchange of genetic information from one taxon to another would remain, either between species or subspecies.

Based on phylogenetic tree reconstructions we distinguish either seven or nine major mtDNA clades. However, based on the comparison of genetic distances the division into nine clades shows no overlap of inter- and intra-clade distances and seems to be more appropriate. Both assignments reflect geographic regions rather than nominal species (Figs. 2.2 and 2.3). Monophyly of most clades is not well supported and irrespective of the number of clades, our results clearly show that cyt *b* sequence information does not allow any taxonomic inferences. However, based on descriptions of respective holotypes (Schwarz, 1926; Napier, 1981; Groves, 2001), the nine major mtDNA clades fall into the geographic range of the following taxa: C1 = *C. tantalus*, C2 = *C. aethiops*, C3 = *C. cynosuros*, C4 = *C. p. pygerythrus*, C5 = *C. djamdjamensis*, C6 = *C. p. hilgerti*, C7 = *C. sabaeus*, and C8 = *C. p. rufoviridis.* Since for *C. tantalus* a type locality is not available and no taxon has been described from the region of Ghana, Burkina Faso or Togo, the western phenotypic *tantalus* clade (C9) cannot be referred to the geographic range of any previously described taxon. However, Schwarz (1926)

already mentioned a phenotypically different form from Togo which remained undescribed and was not recognized by others (e.g., Booth, 1956; Hill, 1966).

While the holotype of *matschiei* has been phenotypically assigned to *C. aethiops* by most authors (Hill, 1966; Napier, 1981; Groves, 2001), type specimens of *ellenbecki* have been either referred to representatives of *C. pygerythrus* (Napier, 1981; Groves, 2001) or *C. aethiops* (Hill, 1966; Dandelot & Prévost, 1972). Based on observed phenotypic characters we assigned type specimens of both *ellenbecki* (sample IDs 528 and 567) and *matschiei* (sample ID 530) to *C. aethiops* (Fig. 2.1), which is supported by our mtDNA results. Therefore both taxa might represent synonyms of *C. aethiops* based on our findings. Schwarz (1926) explained the distinct characters of the holotype of *C. djamdjamensis* with a local adaption to the harsh mountain climate and listed it as synonym for *hilgerti*. Although we found the main distinguishing features of the holotype to be characteristic of *C. djamdjamensis*, the haplotype of the holotype falls into the *C. aethiops* clade and does not cluster with the distinct *C. djamdjamensis* lineage from the Bale Mountains National Park. This indicates that the holotype of *djamdjamensis* possibly represents a hybrid between *C. djamdjamensis* and *C. aethiops*.

Based on a previous study *C. sabaeus* from St. Kitts originates most probably from Senegal or Gambia (van der Kuyl 1996). In our study we included the cyt *b* sequence of the same reference individual that was used in the study by van der Kuyl (1996; sample ID IV, Table S2.I), and found that this sample clusters together with other samples from Senegal and Mauretania and are not intermingled with samples from Ghana and Burkina Faso (Fig. 2.2). Therefore our data support the hypothesis that Caribbean green monkeys originate from Senegal or adjacent countries of the West African coast.

Compared to previous reports we found some differences in geographic positions of species borders and contact zones. In West Africa Booth (1956) described that the border between *C. sabaeus* and *C. tantalus* follows the Volta and the White Volta River in Ghana. We found the easternmost sample of *C. sabaeus* in Krachi, which is east of the White Volta River (sample ID 553) in Ghana. As for this sample there is no discordance between phenotype and mtDNA, we assume that the border between *C. sabaeus* (C7) and the western *tantalus* clade (C9) possibly follows the Oti River and not the White Volta River in Ghana and Burkina Faso. Several authors mentioned an exceptional

C. tantalus population west of the Volta River on the Accra plains in South Ghana (Booth, 1956, 1958; Hill, 1966; Napier, 1981; Kingdon, 1997), which could be confirmed in our study based on phenotypic data. However, the mtDNA sequences of these *C. tantalus* individuals fall into the *C. sabaeus* clade (C7, Fig. 2.3). Since no individuals with *sabaeus* phenotypes have been found in this area, historic introgressive hybridization is the most probable explanation for the discordance between phenotype and mtDNA of the *C. tantalus* population. Concerning the *C. tantalus* clade in Ghana, Burkina Faso and Togo (C9), future studies should consider samples from Benin and Nigeria, especially east and west of the Niger River, to delimitate the geographical range of this western *C. tantalus* clade and to test if the mtDNA border between the western (C9) and eastern *C. tantalus* (C1) clades follows the Niger River (Fig. 2.3).

In Ethiopia, mtDNA of phenotypes from all three species *C. aethiops*, *C. djamdjamensis* and *C. pygerythrus* cluster together in the clade from South Ethiopia (C2, Fig. 2.3), which indicates that hybridization has occurred and possibly still occurs between all three species in South Ethiopia. Groves (2001) mentioned a possible boundary between *C. aethiops* and *C. pygerythrus* between Lake Shala and Lake Zwai. Our phenotypic data provide evidence that the contact zone between *C. aethiops* and *C. pygerythrus* is about 200 km further to the south close to Lake Abaya, because we found phenotypes of *C. aethiops* and *C. pygerythrus* as well as intermediate forms in this area (sample ID 391, Fig. 2.1), and no phenotypes of *C. pygerythrus* further to the north. Based on phenotypic characters we suggest that individuals from Bubbe Kersa and Gossa (sample IDs 433 and 436) are potential hybrids between *C. djamdjamensis* and *C. aethiops*. MtDNA of samples from those individuals cluster in the *aethiops* clade (C2), which supports the assumption that hybridization occurs between *C. aethiops* and *C. djamdjamensis* in this area. Interestingly, mtDNA of the holotype of *djamdjamensis* (sample ID 529), which was collected close to Bubbe Kersa and Gossa in Abera in 1900, also represents a putative hybrid (Figs. 2.2 and 2.3). These results do not only provide evidence for ongoing hybridization among *C. aethiops* and *C. djamdjamensis*, but also indicate that hybridization already occurred more than 100 years ago in this area. In concordance with Shimada (2002) samples of *C. aethiops* from Woliso in Southwest Ethiopia cluster together with samples of *C. pygerythrus* from Uganda and Kenya in

the *C. tantalus* clade (C1, Figs. 2.2 and 2.3). Since we did not observe *C. tantalus* phenotypes in Ethiopia ongoing hybridization between *C. aethiops* and *C. tantalus* in this area is unlikely and has not been reported yet. The White Nile River was mentioned as possible barrier between *C. aethiops* and *C. tantalus*, but no reliable data about the distribution of *C. aethiops* and *C. tantalus* in this region is available (Lernould, 1988; Engelberger, 2010) (Fig. 2.3). We found further indication for hybridization between the *C. p. rufoviridis* (C8) and the *C. tantalus* (C1) clade in Uganda and Kenya, as we detected phenotypes of *C. pygerythrus* but mtDNA of *C. tantalus* in this region (C1, Fig. 2.3). Our findings confirm Napier's (1981) assumption of hybridization between *C. tantalus*, *C. pygerythrus*, *C. aethiops* and *C. djamdjamensis* in East Africa, who assumed a broad hybrid zone spreading from Uganda northeastwards to Harar in Ethiopia.

Since we do not have information on phenotypes of individuals from Zambia, we cannot exclude that incongruences within *C. cynosuros* and *C. pygerythrus* are simply caused by wrong taxonomic determination of the specimens. Thus, *C. pygerythrus* may be distributed further to the North and *C. cynosuros* further to the east than previously believed (Fig. 2.3). Denser sampling of *C. pygerythrus* and *C. cynosuros* in Zambia is needed to delineate their geographical ranges and to study whether inter-specific gene flow occurs in a respective contact zone. Furthermore additional samples from Southern Africa would help to clarify paraphyletic relationships within the widely distributed species *C. pygerythrus* stretching from Ethiopia to South Africa.

The analysis of the complete cyt *b* gene has been successfully used to reveal the phylogeny of several primates and mammals in general (Castresana, 2001; Agnarsson & May-Collado, 2008; Roos et al., 2008; Thinh et al., 2010; Tobe et al., 2010). This was not possible for *Chlorocebus*. Although analyses revealed several mtDNA clades, we were not able to resolve phylogenetic relationships within the genus. While additional samples from Southern Africa might contribute to a better resolution of phylogenetic relationships within African green monkeys, weak statistical support and consequential uncertainties in basal relationships might also be an indication for the divergence of main lineages within a short time period (Zinner et al., 2009b). Recurrent gene flow among parapatric species, triggered by periodic retractions and expansions of populations in response to Pleistocene climate changes, is another possible reason for

the ambiguous relationships, as it has been found in several African savanna mammals including primates (e.g., *Papio*; Arctander et al., 1999; Flagstad et al., 2001; Muwanika et al., 2003; Lorenzen et al., 2007; Zinner et al., 2009b). The appearing of nine mtDNA clades at least indicates that *Chlorocebus* has experienced certain periods of geographic isolation in its evolutionary history. However, to test if the phylogeography of African green monkeys has been influenced by Pleistocene climate oscillations as suggested for other savanna mammals, further analyses of longer mtDNA sequences, ideally of full mtDNA genomes as well as of nuclear sequences from multiple independent genetic loci are necessary.

CONCLUSION

Our study indicates the importance of dense taxon sampling for revealing the genetic diversity of African green monkeys. It also shows that mtDNA genomes of only few taxa do not effectively reflect the diversity of this species complex. The study of further mtDNA markers or complete mtDNA genomes as well as of nuclear DNA markers from a sample set that represents the diversity of African green monkeys can now be used to improve support of basal relationships and help to obtain a clearer picture of the phylogeography of African green monkeys.

Although our data set includes samples from most African green monkey taxa, subsequent studies should consider further samples from Nigeria, South Sudan and Southeast Africa to provide additional information on species borders in those regions (question marks in Fig. 2.3B). In general, we found that the mtDNA diversity of African green monkeys does not conform to any of the suggested classifications and also that species distributions might need revisions. However, since we found evidence of introgressive hybridization in almost all contact zones between species, mtDNA diversity cannot be regarded as equivalent to species diversity and the analysis of maternally inherited markers alone is not appropriate to delimit species. Therefore we do not consider any taxonomic changes here and advertise studies of nuclear markers to clarify the taxonomic status of the obtained mtDNA clades and the possible impact of hybridization on the mtDNA phylogeny of African green monkeys. Nevertheless, since our data present genetic evidence for the distinctiveness of *C. djamdjamensis* from the

Bale Mountains NP and further confirm ongoing hybridization with *C. aethiops*, more attention should be paid to the conservation of this endemic species and to the protection of its restricted habitat.

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Supplemental material

Table S2.I Taxon identity, sampling sites and GenBank accession numbers of the cytochrome *b* sequences of 126 *Chlorocebus* samples used in this study. Taxa are abbreviated with three initial letters and stars indicate type specimens.

ID	Taxon	Country	Sample site	Longitude Latitude	Nature	Institution, Collection No.	GenBank Cyt *b*
I	pyg	Republic of South Africa	-	-		GenBank	EF597500
II	pyg	Tanzania/ Kenya	-	-		GenBank	EF597501
III	tan	Central African Republic	-	-		GenBank	EF597502
IV	sab	Senegal	-	-		GenBank	EF597503
1	aet	Eritrea	Fil Fil	15.61720 38.97075	hair	DPZ	JX983734
2	aet	Eritrea	Fil Fil	15.61720 38.97075	hair	DPZ	JX983735
3	aet	Eritrea	Faulena	14.78750 37.99580	hair	DPZ	JX983733
8	aet	Eritrea	Anseba River	15.70652 38.62617	hair	DPZ	JX983730
9	aet	Eritrea	Mt. Bizen	15.33333 39.06194	hair	Zoo Asmara	JX983731
11	aet	Eritrea	Mt. Bizen	15.33333 39.06194	hair	Zoo Asmara	JX983732
26	tan	Central African Republic	-	-	hair	Museum Besancon	JX983846
64	pyg	Republic of South Africa	Loskop Dam NR	-25.42158 29.295536	feces	DPZ	JX983769
65	pyg	Republic of South Africa	Loskop Dam NR	-25.42158 29.295536	feces	DPZ	JX983770
70	cyn	Zambia	Kasanka NP	-12.57276 30.23332	feces	DPZ	JX983756
72	pyg	Zambia	Shiwa N'gandu	-11.19677 31.73892	feces	DPZ	JX983752
75	pyg	Republic of South Africa	Bedford	-32.68334 26.08350	skin	ZFMK 61.269	JX983771
78	pyg	Republic of South Africa	Bedford	-32.68334 26.08350	skin	ZFMK 62.3	JX983772
91	cyn	Zambia	Fibwe	-12.59050 30.25201	feces	DPZ	JX983755
101	cyn	Zambia	Chunga	-15.04440 25.99910	feces	DPZ	JX983754
102	tan	Nigeria	Gashaka Gumpi NP	11.19677 31.73891	feces	DPZ	JX983843
103	tan	Nigeria	Gashaka Gumpi NP	11.19677 31.73891	feces	DPZ	JX983844
104	tan	Nigeria	Gashaka Gumpi NP	11.19677 31.73891	feces	DPZ	JX983845
118	sab	Senegal	Niokolo Koba NP	13.07536 12.72239	feces	DPZ	JX983827
120	sab	Senegal	Niokolo Koba NP	13.02577 13.23736	feces	DPZ	JX983828
138	sab	Ghana	Bui NP	8.29083 -2.28465	feces	DPZ	JX983804

ID	Taxon	Country	Sample site	Longitude Latitude	Nature	Institution, Collection No.	GenBank Cyt *b*
139	sab	Ghana	Bui NP	8.29114 -2.28431	feces	DPZ	JX983805
142	sab	Ghana	Bui NP	8.29114 -2.28431	feces	DPZ	JX983806
143	sab	Ghana	Bui NP	8.29090 -2.28388	feces	DPZ	JX983807
144	sab	Ghana	Bui NP	8.29126 -2.28315	feces	DPZ	JX983808
145	sab	Ghana	Bui NP	8.29083 -2.28465	feces	DPZ	JX983809
146	sab	Ghana	Bui NP	8.29083 -2.28465	feces	DPZ	JX983810
153	sab	Ghana	Mole NP	9.26008 -1.86058	feces	DPZ	JX983811
158	sab	Ghana	Mole NP	9.26058 -1.86147	feces	DPZ	JX983812
160	sab	Ghana	Mole NP	9.25876 -1.84714	feces	DPZ	JX983813
161	sab	Ghana	Mole NP	9.25876 -1.84714	feces	DPZ	JX983814
165	sab	Ghana	Mole NP	9.25876 -1.84714	feces	DPZ	JX983815
166	sab	Ghana	Mole NP	9.25183 -1.86105	feces	DPZ	JX983816
167	sab	Ghana	Mole NP	9.25183 -1.86105	feces	DPZ	JX983817
168	sab	Ghana	Mole NP	9.25183 -1.86105	feces	DPZ	JX983818
169	sab	Ghana	Mole NP	9.25183 -1.86105	feces	DPZ	JX983819
170	sab	Ghana	Mole NP	9.25183 -1.86105	feces	DPZ	JX983820
171	sab	Ghana	Mole NP	9.25183 -1.86105	feces	DPZ	JX983821
172	sab	Ghana	Mole NP	9.25183 -1.86105	feces	DPZ	JX983822
191	tan	Ghana	Kalapka RR	6.45293 0.38055	feces	DPZ	JX983839
192	tan	Ghana	Kalapka RR	6.45293 0.38055	feces	DPZ	JX983840
193	tan	Ghana	Kalapka RR	6.45293 0.38055	feces	DPZ	JX983841
203	tan	Ghana	Shai Hills RR	5.89777 0.06897	feces	DPZ	JX983823
208	tan	Ghana	Shai Hills RR	5.89001 0.04382	feces	DPZ	JX983824
212	tan	Burkina Faso	Buffle Rouge	11.30183 1.04397	feces	DPZ	JX983836
213	tan	Burkina Faso	Buffle Rouge	11.30183 1.04397	feces	DPZ	JX983837
215	tan	Burkina Faso	Buffle Rouge	11.30183 1.04397	feces	DPZ	JX983838
223	tan	Burkina Faso	Park D'Arly	11.60094 1.39187	feces	DPZ	JX983830
224	tan	Burkina Faso	Park D'Arly	11.60094 1.39187	feces	DPZ	JX983831
225	tan	Burkina Faso	Park D'Arly	11.60094 1.39187	feces	DPZ	JX983832

ID	Taxon	Country	Sample site	Longitude Latitude	Nature	Institution, Collection No.	GenBank Cyt *b*
226	tan	Burkina Faso	Park D'Arly	11.58281 1.46103	feces	DPZ	JX983833
227	tan	Burkina Faso	Park D'Arly	11.58281 1.46103	feces	DPZ	JX983834
228	tan	Burkina Faso	Park D'Arly	11.58087 1.45781	feces	DPZ	JX983835
233	tan	Burkina Faso	Park D'Arly	11.58087 1.45781	feces	DPZ	JX983829
239	sab	Burkina Faso	Ranch Nazinga	11.16182 -1.60967	feces	DPZ	JX983793
241	sab	Burkina Faso	Ranch Nazinga	11.13514 -1.61265	feces	DPZ	JX983794
246	sab	Burkina Faso	Ranch Nazinga	11.15594 -1.61445	feces	DPZ	JX983795
247	sab	Burkina Faso	Ranch Nazinga	11.14870 -1.62334	feces	DPZ	JX983796
248	sab	Burkina Faso	Ranch Nazinga	11.14870 -1.62334	feces	DPZ	JX983797
250	sab	Burkina Faso	Ranch Nazinga	11.14870 -1.62334	feces	DPZ	JX983798
251	sab	Burkina Faso	Ranch Nazinga	11.14870 -1.62334	feces	DPZ	JX983799
254	sab	Burkina Faso	Ranch Nazinga	11.15547 -1.61030	feces	DPZ	JX983800
255	sab	Burkina Faso	Ranch Nazinga	11.15547 -1.61030	feces	DPZ	JX983801
256	sab	Burkina Faso	Ranch Nazinga	11.15547 -1.61030	feces	DPZ	JX983802
264	sab	Burkina Faso	FC Deux Bale	11.55079 -2.95757	feces	DPZ	JX983792
268	sab	Burkina Faso	Comoe Leraba NP	9.90260 -4.65454	feces	DPZ	JX983778
269	sab	Burkina Faso	Comoe Leraba NP	9.90260 -4.65454	feces	DPZ	JX983779
271	sab	Burkina Faso	Comoe Leraba NP	9.90260 -4.65454	feces	DPZ	JX983780
272	sab	Burkina Faso	Comoe Leraba NP	9.84896 -4.62302	feces	DPZ	JX983781
273	sab	Burkina Faso	Comoe Leraba NP	9.84896 -4.62302	feces	DPZ	JX983782
275	sab	Burkina Faso	Comoe Leraba NP	9.84896 -4.62302	feces	DPZ	JX983783
277	sab	Burkina Faso	Comoe Leraba NP	9.85291 -4.61415	feces	DPZ	JX983784
279	sab	Burkina Faso	Comoe Leraba NP	9.86992 -4.65626	feces	DPZ	JX983785
281	sab	Burkina Faso	Comoe Leraba NP	9.86992 -4.65626	feces	DPZ	JX983786
283	sab	Burkina Faso	Comoe Leraba NP	9.86207 -4.66974	feces	DPZ	JX983787
284	sab	Burkina Faso	Comoe Leraba NP	9.86207 -4.66974	feces	DPZ	JX983788
285	sab	Burkina Faso	Comoe Leraba NP	9.86207 -4.66974	feces	DPZ	JX983789
286	sab	Burkina Faso	Comoe Leraba NP	9.86207 -4.66974	feces	DPZ	JX983790
293	sab	Burkina Faso	Comoe Leraba NP	9.77530 4.60271	feces	DPZ	JX983791

ID	Taxon	Country	Sample site	Longitude Latitude	Nature	Institution, Collection No.	GenBank Cyt *b*
316	aet	Ethiopia	Tana	11.68854 37.32800	feces	DPZ	JX983749
317	aet	Ethiopia	Tana	11.68854 37.32800	feces	DPZ	JX983750
319	dja	Ethiopia	Harenna, Bale Mts. NP	6.75993 39.73711	feces	DPZ	JX983758
320	dja	Ethiopia	Harenna, Bale Mts. NP	6.75993 39.73711	feces	DPZ	JX983759
323	dja	Ethiopia	Harenna, Bale Mts. NP	6.75993 39.73711	feces	DPZ	JX983760
330	aet	Ethiopia	Lake Awassa	7.04809 38.46210	feces	DPZ	JX983741
331	aet	Ethiopia	Lake Awassa	7.04809 38.46210	feces	DPZ	JX983742
373	aet	Ethiopia	Sodore	8.40321 39.39247	feces	DPZ	JX983740
391	aet/pyg	Ethiopia	Arba Minch	6.03850 37.57189	feces	DPZ	JX983739
400	aet	Ethiopia	Jinka	5.72704 36.64093	feces	DPZ	JX983746
421	pyg	Ethiopia	Yabello	4.89596 38.07043	feces	DPZ	JX983766
433	dja/aet	Ethiopia	Bubbe Kersa	6.14111 38.73954	feces	DPZ	JX983761
436	dja/aet	Ethiopia	Gossa	6.28349 38.67784	feces	DPZ	JX983762
438	aet	Ethiopia	Jimma	7.69243 36.80676	feces	DPZ	JX983744
439	aet	Ethiopia	Jimma	7.69243 36.80676	feces	DPZ	JX983745
451	aet	Ethiopia	Bonga	7.26645 36.23131	feces	DPZ	JX983743
464	aet	Ethiopia	Woliso	8.53230 37.98187	feces	DPZ	JX983751
484	aet	Ethiopia	Menegasha	8.96569 38.52645	feces	DPZ	JX983747
485	aet	Ethiopia	Menegasha	8.96569 38.52645	feces	DPZ	JX983748
496	pyg	Ethiopia	Sof Omar	6.91205 40.84583	feces	DPZ	JX983764
503	pyg	Tanzania	Iringa	-7.279220 35.738340	feces	DPZ	JX983773
504	pyg	Kenya	Sukari Ranch	-1.25000 37.10000	skin	NMK SUK3	JX983767
507	pyg	Kenya	Charangani	0.98333 35.21667	skin	NMK 4836	JX983851
513	pyg	Kenya	Kilgoris	-1.00541 34.87197	skin	NMK 6783	JX983850
520	sab	Liberia	Gola Country	7.44317 -10.77779	skin	ZSM 5	JX983825
522	pyg	Tanzania	Fundo Island, Pemba	-5.05264 39.64780	tissue	MfN 26	JX983774
524	pyg	Tanzania	Bukoba	-1.32404 31.80739	skin	MfN 9091	JX983775
528	ell*	Ethiopia	Suksuk River	7.78830 38.67257	skin	MfN 35504	JX983736
529	dja*	Ethiopia	Abera, 3200m	6.44189 38.47116	skin	MfN 35505	JX983757

ID	Taxon	Country	Sample site	Longitude Latitude	Nature	Institution, Collection No.	GenBank Cyt *b*
530	mat*	Ethiopia	Malo(Naja), Omo River	6.58333 36.55000	tissue	MfN 35509	JX983737
535	sab	Mauretania	Podor, Senegal River	16.54844 -14.24304	tooth	MfN 40413	JX983826
536	tan	Togo	Bassari	9.23291 0.76747	skin	MfN 77410	JX983842
539	tan	Cameroon	Dschang District	5.39621 9.87775	tooth	MfN 87395	JX983848
540	pyg	Kenya	Guaso Njiro River	0.31035 37.19262	tissue	MfN 87411	JX983768
545	pyg	Somalia	Bardera	2.33364 42.28266	tissue	MfN 87445	JX983765
546	aet	Ethiopia	Roba Butta	6.81667 40.76667	tooth	MfN 87449	JX983763
549	pyg	Tanzania	Mkulwe	-8.58079 32.31928	tooth	MfN 87461	JX983776
553	sab	Ghana	Kratshi/Krachi	7.80000 -0.05000	tissue	MfN 87496	JX983803
560	pyg	Uganda	W of Lake Albert	2.21534 31.28184	tissue	MfN 87511	JX983849
561	tan	Cameroon	Lake Chad	12.94512 14.33129	tissue	MfN 87512	JX983847
563	pyg	Tanzania	Pangani River, middle course	-5.16886 38.35409	tissue	MfN 87557	JX983777
566	cyn	Angola	Cubal River, Benguela	-12.81594 13.65009	tooth	MfN 87998	JX983753
567	ell*	Ethiopia	Suksuk River	7.78830 38.67257	tissue	MfN 88754	JX983738

NP= National Park, NR= Nature Reserve, FC= Forêt Classée, RR= Resource Reserve.
DPZ= Deutsches Primatenzentrum, Goettingen, Germany
ZFMK= Zoologisches Forschungsmuseum Alexander Koenig, Bonn, Germany
NMK= National Museums of Kenya, Nairobi, Kenya
ZSM= Zoologische Staatssammlung München, Munich, Germany
MfN= Museum für Naturkunde, Berlin, Germany
aet = *aethiops*, cyn = *cynosuros*, dja = *djamdjamensis*, ell = *ellenbecki*, mat = *matschiei*, pyg = *pygerythrus*, sab = *sabaeus*, tan = *tantalus*

Table S2.II Primer sequences, product sizes and annealing temperatures (T_a) used for the amplification of the cytochrome *b* gene in this study.

Primer ID	Sequence (5'-3')	Size [bp]	T_a [°C]
2 overlapping fragments			
cytbAfw	CCACCGTTGTACTTCAACTAC	681	62
cytbArv	TTGTCTGAGTCTGATGAGATTC		62
cytbBfw	CCACCCTTTCACGATTCTTCA	664	62
cytbBrv	TAGTTTACAAGACTAGTGTATTAG		62
4 overlapping fragments			
cytbA1fw	CCACCGTTGTACTTCAACTAC	369	62
cytbA1rv	CAGGTTTTTAGGAGAAGGAATG		62
cytbA2fw	GGCGCCTCCATATTTTTCATC	334	62
cytbA2rv	TTGTCTGAGTCTGATGAGATTC		62
cytbB1fw	CCACCCTTTCACGATTCTTCA	382	62
cytbB1rv	ATGAGGATTGATAGGAAGAGTG		62
cytbB2fw	CCCTCCACACATCAAACCAG	390	62
cytbB2rv	TAGTTTACAAGACTAGTGTATTAG		62
cytbB2fw4	YCCACACATCAAACCAG	322	52
cytbB2rv4*	TAGAATGCCAGTTTTGGG		52
6 overlapping fragments			
cytbM1fw	CCACCGTTGTACTTCAACTAC	269	62
cytbM1rv	CGAATGATTCAGCCGTGGTTT		62
cytbM2fw	CAGACACCTCTTCTGCCTTC	262	62
cytbM2rv	GTTGCYCCYCAGAATGATATT		62
cytbM3fw	ATAGCAACAGCYTTYATAGGCT	252	62
cytbM3rv	GTGTAGAAACAGCAGATGGAC		62
cytbM4fw	CGATTCTTCACCCTACACTTTA	283	62
cytbM4rv	CTGGTTTGATGTGTGGRGGG		62
cytbM5fw	CTRAACGACCCAGACAACTA	272	62
cytbM5rv	CTTCCGATCCAGGTGAGGG		62
cytbM6fw	AGCATAATATTCCGCCCACTTA	242	62
cytbM6rv	TAGTTTACAAGACTAGTGTATTAG		62

* (Naidu et al., 2012)

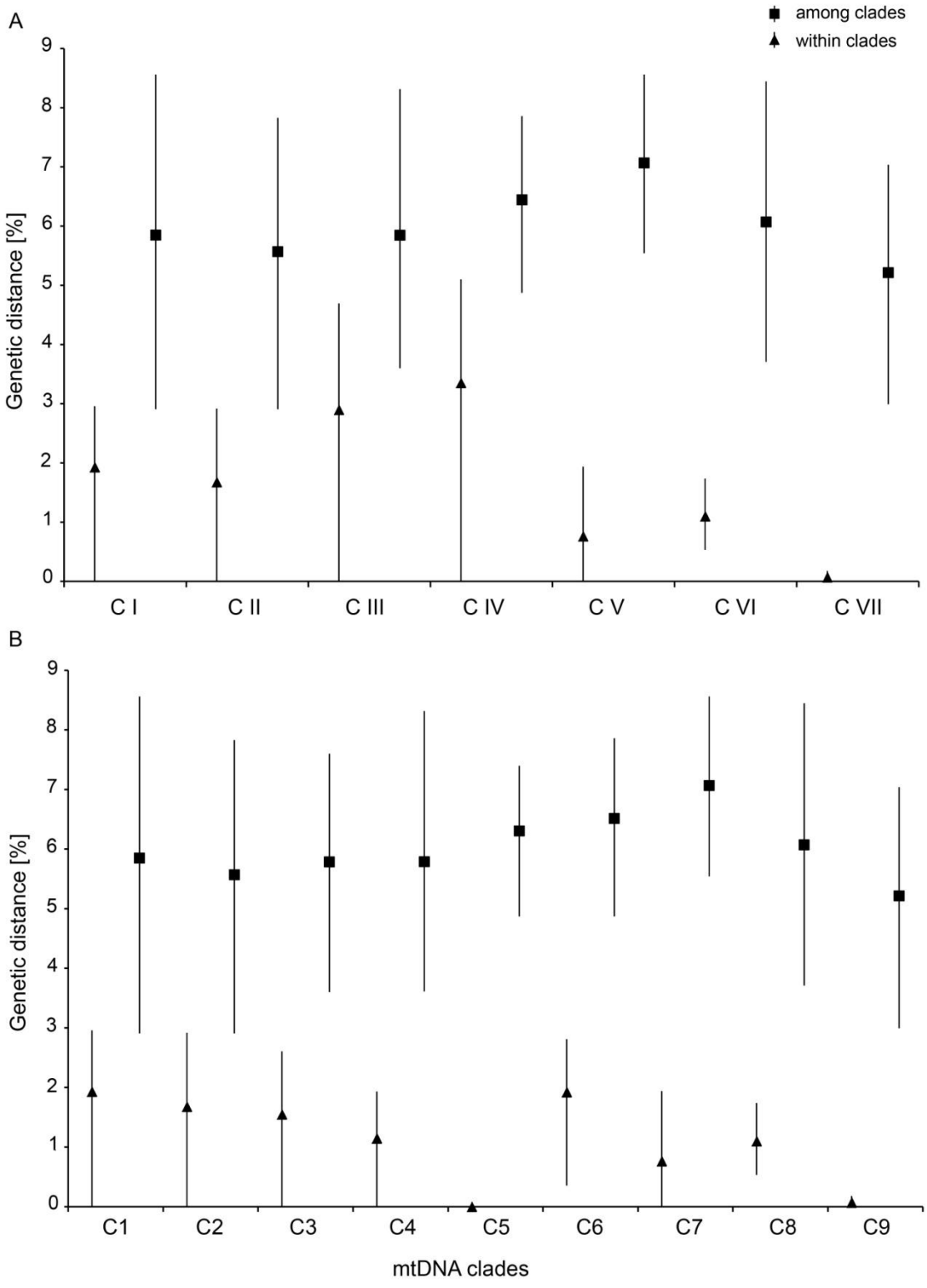

Fig. S2.1 Ranges of genetic distances (min, mean, max) within and among mtDNA clades in percent. Depicted are intra- and inter-clade distances using a classification of (A) seven (C I-VII) and (B) nine (C1-9) mtDNA clades.

CHAPTER 3

Discordance between mtDNA variation and morphotype distribution in African green monkeys (*Chlorocebus*): evidence of cryptic variation or introgressive hybridization?

Tanja Haus[1,2,3], Christian Roos[2,4] and Dietmar Zinner[1]

1 Cognitive Ethology Laboratory, German Primate Center, Leibniz Institute for Primate Research, Göttingen, Germany

2 Primate Genetics Laboratory, German Primate Center, Leibniz Institute for Primate Research, Göttingen, Germany

3 Centre of Biodiversity and Sustainable Land Use, University of Göttingen, Göttingen, Germany

4 Gene Bank of Primates, German Primate Center, Leibniz Institute for Primate Research, Göttingen, Germany

CR and DZ contributed equally

Short title: *Chlorocebus* introgressive hybridization

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Abstract

A recent molecular genetic study on mitochondrial DNA (mtDNA) indicated several paraphylies within the phylogeny of African green monkeys (*Chlorocebus*) suggesting contemporary and/or ancient introgressive hybridization among almost all species. Most paraphyletic relationships support previous observations on on-going hybridization among species in East Africa, but supposed hybridization in the northern part of the contact zone in West Africa could not be detected. However, based on the analysis of solely mtDNA no final conclusions can be drawn on (ancient) introgression events. In this study we analysed two Y chromosomal (Y-chr) markers of 30 African green monkey samples and compared genetic relationships to those based on published mtDNA data. In concordance with mtDNA, we found two distinct and not closely related Y-chr haplotypes within the range of *C. tantalus* suggesting possible cryptic genetic variation rather than ancient introgressive hybridization. Accordingly, *C. tantalus* possibly represents two different and previously unrecognized taxa. In contrast, Y-chr data produce monophyletic relationships within *C. pygerythrus* from East Africa suggesting that mtDNA paraphylies within *C. pygerythrus* are most likely the result of ancient introgressive hybridization and subsequent cytonuclear extinction of a former taxon. However, based on Y-chr DNA we found no evidence of supposed hybridization among *C. sabaeus* and *C. tantalus* in the northern part of the contact zone in West Africa. Our results accentuate the importance of the analysis of nuclear data in addition to mtDNA, to obtain a more complete picture on evolutionary processes and potential outcomes of hybridization with respect to genetic and species diversity.

Keywords: primates, ancient hybridization, nuclear swamping, outcomes of hybridization

INTRODUCTION

Within the last two decades an increasing number of molecular genetic studies, especially those based on mitochondrial DNA (mtDNA), revealed evidence of hybridization in diverse animal taxa (Mallet, 2005; Zinner et al., 2011a; Abbott et al., 2013). Introgressive hybridization, i.e. the transmission of gene material of one species into the genome of another (closely related) species, accounts for a large proportion of detected reticulated phylogenies and paraphyletic relationships (Mallet, 2005). Due to the often inviable or sterile heterogametic sex in hybrids, in mammals introgressive hybridization causes predominantly the transmission of the maternally inherited mitochondrial genome (Orr, 1997; Mallet, 2005; Schilthuizen et al., 2011). However, depending on the dispersing sex and the effective population size of involved taxa, backcrossing might occur preferentially with only one of the parental species, which may lead to nuclear swamping or to mitochondrial and Y chromosomal capture (Zinner et al., 2011a).

Evidence of frequent introgressive hybridization was also found in diverse African savannah mammals and has been attributed to recurrent changes in the extent and distribution of the savannah biome and hence in population distributions and the formation of secondary contact zones due to climatic changes within the last million years (e.g., hartebeests (Flagstad et al., 2001), warthogs (Muwanika et al., 2003), baboons (Zinner et al., 2009b; Keller et al., 2010)). Baboons (*Papio*) are widely distributed in Sub-Saharan African savannahs and on-going hybridization occurs in most places where ranges of two species meet (Tung et al., 2008; Jolly et al., 2011; Charpentier et al., 2012). Moreover, Zinner et al. (2009b) found discordant patterns among mitochondrial DNA and morphotypes in regions where no current hybrid zone exist. Like in most mammalian taxa, in baboons males are predominantly the dispersing sex. Therefore they explained discordant patterns in baboons by nuclear swamping through male-mediated introgressive hybridization. In some of these cases introgression presumably led to the cytonuclear extinction of historic baboon taxa or populations, whose mitochondrial genomes persist only as a trace in extant species (Zinner et al., 2009b; Keller et al., 2010).

Similar to baboons, African green monkeys of the genus *Chlorocebus* constitute parapatric species which inhabit sub-Saharan savannahs (Hill, 1966; Lernould, 1988;

Kingdon, 1997). Based on morphological data hybridization has been assumed between most of the species in East Africa (Napier, 1981; Kingdon, 1997; Mekonnen et al., 2012). Furthermore, a recent study of mitochondrial sequence data including samples from almost all contact zones throughout the genus' range found several discordant phylogenetic patterns suggesting that introgressive hybridization might be common among all African green monkeys (Haus et al., 2013). Most of these discordances support previous observations on on-going hybridization in contemporary contact zones between *C. aethiops* and *C. djamdjamensis* and between *C. aethiops* and *C. pygerythrus* in Ethiopia, as well as between *C. tantalus* and *C. pygerythrus* in Uganda and Kenya (Fig. 3.1a; (Napier, 1981; Kingdon, 1997; Haus et al., 2013). In addition, Haus et al. (2013) found some inconsistencies among morphology and mtDNA beyond current contact zones, which might be indicative for previously unrecognized ancient introgression events like in baboons. Their analysis revealed two distinct clades within the range of *C. tantalus*: a western clade including samples from Ghana, Burkina Faso and Togo, and an eastern clade with samples ranging from Nigeria to Uganda and Kenya (Fig. 3.1a). Similarly, *C. pygerythrus* represents a paraphyletic taxon with distinct clades found in Southeast Ethiopia and Somalia, in Kenya and Tanzania, and in Southern Africa (Fig. 3.1a; Haus et al., 2013). Since these clades represent geographically monophyletic clades, random sorting failures, for example incomplete lineage sorting, are unlikely to have caused respective discordances (Funk & Omland, 2003; Avise, 2004). Alternatively, a possible reason for the discordant patterns might be nuclear swamping and subsequent cytonuclear extinction through ancient introgression as it has been suggested for some baboon taxa.

Conflicting information exist on the occurrence of hybridization between *C. sabaeus* and *C. tantalus*. Both taxa seem to be separated by the White Volta and the Oti River in Ghana (Fig. 3.1; Booth, 1956, 1958; Haus et al., 2013). However, hybridization has been assumed to occur in the northern part of the contact zone in Burkina Faso, where confluents of the Volta River most likely do not represent geographical barriers for the monkeys (Fig. 3.1a; Lernould, 1988). Contrary to this assumption, no indication for hybridization was found in this part of the contact zone, but in a single population in Southern Ghana based on mtDNA data (Fig. 3.1a; Haus et al.,

2013). However, hybridization processes like Y chromosomal capture cannot be detected by the analysis of mtDNA.

Based on morphological characters and mitochondrial sequence information alone no final conclusions can be drawn on processes that have led to genetic patterns within African green monkeys. Therefore, in this study we analyse two Y-chr markers of 30 African green monkey samples and compare results to genetic relationships based on previously published mitochondrial DNA sequences. We focus 1) on potential ancient introgression events, which might have led to paraphyletic relationships within *C. tantalus* and within *C. pygerythrus* from East Africa; and 2) on supposed hybridization in the West African contact zone between *C. sabaeus* and *C. tantalus* (Fig. 3.1b). 1) If certain mtDNA clades within *C. tantalus* and *C. pygerythrus* represent relict mitochondrial genomes as a result of ancient introgression, we suppose a pattern of Y-chr DNA that is concordant with morphological data of respective species, but not with mtDNA; and 2) if introgressive hybridization occurred or still occurs among African green monkey species in the West African contact zone we expect that our analysis will reveal discordant genetic relationships between Y-chr and mtDNA data, and thus, paraphyletic patterns within *C. sabaeus* or *C. tantalus* with respect to their morphology.

METHODS

Data sampling

To study potential ancient introgressive hybridization within *C. pygerythrus* and *C. tantalus* as well as assumed hybridization between *C. sabaeus* and *C. tantalus*, we used faecal samples collected in Ethiopia, Ghana and Burkina Faso. In Ethiopia we collected faeces of *C. aethiops* and of the *C. pygerythrus* population from Southeast Ethiopia. In Ghana and Burkina we collected samples at nine different sites on both banks of the Volta River and its confluents covering the distribution of the western *C. tantalus* clade as well as the contact zone of *C. sabaeus* and *C. tantalus* (Fig. 3.1b). We preserved faeces in >90% ethanol for at least 24 hours, and after drying, we transferred them for further storage into tubes with silica beads (Nsubuga et al., 2004). Whenever a green monkey was observed sighting we recorded information on morphological characters to determine respective species according to Haus et al. (2013). Furthermore we recorded all geographic localities using GPS (Table 3.1). We additionally used faecal

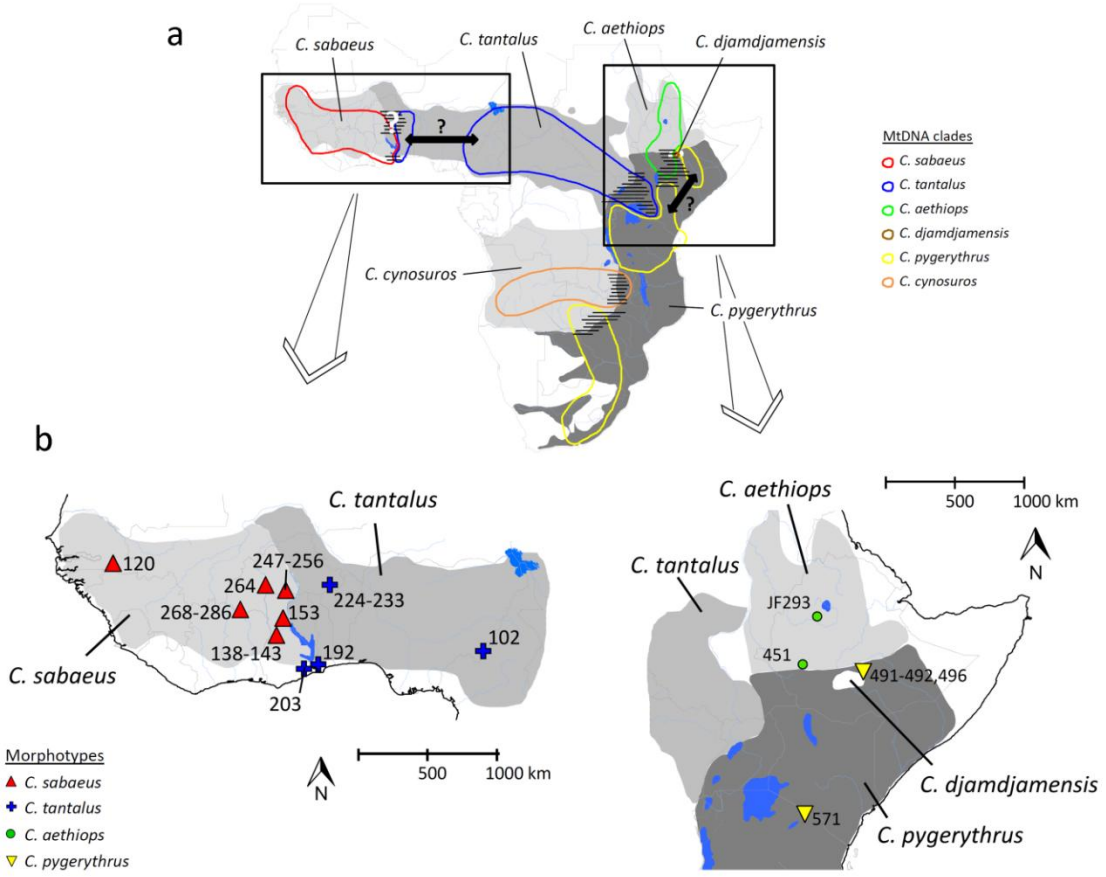

Fig. 3.1 Distribution map of African green monkeys (*Chlorocebus* spp.). a) Distribution of mitochondrial DNA (mtDNA) clades and presumed hybridization in contemporary contact zones (hatched areas) according to Haus et al. (2013). Possible ancient introgression events, which are tested in this study, are indicated with black arrows. Supposed hybridization in the West African contact zone is indicated with a white question mark. b) Collection sites of samples of respective morphotypes from West and East Africa used in this study.

samples of *C. tantalus* (East Nigeria) and *C. sabaeus* (Senegal), which were obtained from other projects in respective countries, and blood samples of *C. sabaeus* from Barbados and of *C. pygerythrus* from Kenya or Tanzania provided by the Paul Ehrlich Institute, Langen, Germany (Fig. 3.1b, Table 3.1).

Data collection was carried out in compliance with respective animal care regulations for the ethical treatment of non-human primates. All faecal samples were collected non-invasively from wild, non-habituated groups without threatening or harming the animals. The blood sample was taken for regular diagnostic checks by veterinarians. The research was conducted according to respective national and international laws and complied with protocols approved by the German Primate Center in Germany, the Ethiopian Wildlife and Conservation Authority (EWCA) in Ethiopia, the

Centre National de la Recherche Scientifique et Technologique (CNRST) in Burkina Faso, and the Forestry Commission (FC) of Ghana, and adhered to the legal requirements of the countries in which the research was conducted.

Extraction, amplification and sequencing of DNA

We extracted total genomic DNA from faecal and blood samples using the QIAamp DNA Stool Mini Kit and the DNeasy Blood & Tissue Kit from Qiagen (Qiagen, Germany), respectively. We applied standard protocols as provided by the company with only minor changes for the extraction of faecal samples (Haus et al., 2013). The extract of the blood samples were stored at 4°C whereas extracts of faecal sample were stored at -20°C in 50 µl aliquots for up to 24 months before further processing.

For the analyses of Y-chr markers samples from only male individuals can be used. Since the collection of faecal samples from unhabituated wild populations does not allow the assignment of samples to certain individuals, information on the individuals' sex was obtained by a gonosomal polymerase chain reaction (PCR) based sexing method (Roos, unpublished data).

We amplified and sequenced fragments of two Y-chr genes: 695 bp of the last intron of the Zinc finger (ZFY) and a 783 bp long fragment of the sex determining region (SRY). Both markers were amplified via two overlapping fragments, each of them measuring around 450-500 bp length, using the primer pairs ZFY-F1: CCTGATTCCAGGGAGTACC and ZFY-R1: AGTAAAGCTTAACTGCACCTAT, ZFY-F2: AGGACAGATTACTATCCTGTG and ZFY-R2: CAGTATGAGTGCTTAATCAAAC, SRY-F1: CTTGAGAATGAATACATTGTCAGGG and SRY-R1: GTATCCCAGCTGCTTGCTG, and SRY-F2: GATCAGAGGCGCAAGATGG and SRY-R2: AGGTCTTTGTAGCCAATGTTACCCG. We used 1 U BiothermTaq 5000 (Genecraft, Germany) in a 30 µl PCR mix (1x reaction buffer, 0.16 mM for each dNTP, 0.33 µM for each primer, and 0.6 mg/ml BSA), with the following thermo cycler conditions: 94°C for 2 min, followed by 60 cycles of 94°C for 1 min, 60°C (SRY) and 58°C (ZFY) for 1 min, 72°C for 1 min, and 72°C for 5 min. All PCR reactions were conducted with at least one PCR blank (HPLC-purified water). We run and checked PCR products on 1-2% agarose gels, and after the excision of DNA fragments of relevant lengths, we purified PCR products with the Qiagen Gel Extraction Kit (Qiagen, Germany). We used the BigDye Terminator Cycle Sequencing Kit (Applied Biosystems, Germany)

and respective forward and reverse primers to run sequences on an ABI 3130 *xL* sequencer. We deposited all sequences in GenBank (Table 3.1).

Table 3.1 Sample identities, collection sites, and GenBank accession numbers of the two Y chromosomal markers SRY and ZFY and the mitochondrial cytochrome *b* gene (cyt *b*).

ID	Species	Country	Location	Longitude Latitude	GenBank accession no. SRY	ZFY	Cyt *b*
451	aet	Ethiopia	Bonga	7.26645 36.23131	X	X	JX983743*
JF293	aet	-	-	-	JF293182§	JF293267§	-
491	pyg	Ethiopia	Sof Omar Caves	6.91205 40.84583	X	X	-
492	pyg	Ethiopia	Sof Omar Caves	6.91205 40.84583	X	X	-
496	pyg	Ethiopia	Sof Omar Caves	6.91205 40.84583	-	-	JX983764*
571	pyg	PEI, TZ/KY	-	-	X	X	X
102	tan	Nigeria	Gashaka Gumti NP	11.19677 31.73892	X	X	JX983843*
225	tan	Burkina Faso	Park D'Arly	11.60094 1.39187	X	X	JX983832*
226	tan	Burkina Faso	Park D'Arly	11.58281 1.46103	X	X	JX983833*
233	tan	Burkina Faso	Park D'Arly	11.58087 1.45781	X	X	JX983829*
224	tan	Burkina Faso	Park D'Arly	11.60094 1.39187	X	X	JX983831*
192	tan	Ghana	Kalapka RR	6.45293 0.38055	X	X	JX983840*
203	tan	Ghana	Shai Hills RR	5.89777 0.06897	-	-	JX983823*
569	sab	PEI, Barbados	-	-	X	X	-
255	sab	Burkina Faso	Ranch Nazinga	11.1554- 1.61030	X	X	JX983801*
256	sab	Burkina Faso	Ranch Nazinga	11.15547 -1.61030	X	X	JX983802*
269	sab	Burkina Faso	Comoe-Leraba NP	9.90260 -4.65454	X	X	JX983779*
143	sab	Ghana	Bui NP	8.29090 -2.28388	X	X	JX983807*

ID	Species	Country	Location	Longitude Latitude	GenBank accession no. SRY	ZFY	Cyt *b*
120	sab	Senegal	Niokolo Koba NP	13.02577 13.23736	X	X	JX983828*
142	sab	Ghana	Bui NP	8.29114 -2.28431	X	X	JX983806*
153	sab	Ghana	Mole NP	9.26008 -1.86058	X	X	JX983811*
286	sab	Burkina Faso	Comoe-Leraba NP	9.86207 -4.66974	X	X	JX983790*
275	sab	Burkina Faso	Comoe-Leraba NP	9.84896 -4.62302	X	X	JX983783*
284	sab	Burkina Faso	Comoe-Leraba NP	9.86207 -4.66974	X	X	JX983788*
139	sab	Ghana	Bui NP	8.29114 -2.28431	X	X	JX983805*
264	sab	Burkina Faso	Deux Bale Foret	11.55079 -2.95757	X	X	JX983792*
247	sab	Burkina Faso	Ranch Nazinga	11.14870 -1.62334	X	X	JX983796*
277	sab	Burkina Faso	Comoe-Leraba NP	9.85291 -4.61415	X	X	JX983784*
285	sab	Burkina Faso	Comoe-Leraba NP	9.86207 -4.66974	X	X	JX983789*
138	sab	Ghana	Bui NP	8.29083 -2.28465	X	X	JX983804*
271	sab	Burkina Faso	Comoe-Leraba NP	9.90260 -4.65454	X	X	JX983780*
268	sab	Burkina Faso	Comoe-Leraba NP	9.90260 -4.65454	X	X	JX983778*

aet=*aethiops*, pyg=*pygerythrus*, tan=*tantalus*, sab=*sabaeus*, PEI=Paul Ehrlich Institute, TZ/KY=Tanzania/Kenya, NP=National Park, RR=Resource Reserve
* Haus et al. (2013)
§ Roos et al. (2011)

Analysis of genetic structures

To compare obtained results of Y-chr data with mtDNA results in African green monkeys, we used published cytochrome *b* (cyt *b*) gene sequences from Haus et al. (2013). We selected cyt *b* sequences from the same samples (or specimens) of which we sequenced Y-chr markers. In cases where mitochondrial sequences for respective specimens were not available we used mitochondrial sequence information from samples that were

collected at the same sites, assuming that animals from the same social groups carry relatively similar mtDNA genomes (Fig. 3.1, Table 3.1).

We assembled and aligned all Y-chr sequences with the program Geneious Pro 5.0.4 (Drummond et al., 2011), and added an additional sequence from GenBank representing *C. aethiops* (Roos et al., 2011; Table 3.1). Furthermore, we used Geneious to prepare two separate alignments, one concatenated alignment of the two Y-chr markers ZFY and SRY, and a second alignment including all cyt *b* sequences. To remove deletions in the Y-chr data set we used the software Gblocks (Talavera & Castresana, 2007) leading to a final length of the Y-chr alignment of 1476 bp, for which we measured variable and parsimony-informative sites with Mega 5.0 (Tamura et al., 2011). To compare genetic patterns of Y-chr and mitochondrial DNA we calculated median-joining networks for both data sets separately, using the computer program Network 4.610 (Bandelt et al., 1999). Finally, we displayed and edited resulting networks with the software Network Publisher.

RESULTS

The samples which we included in our analysis represent morphologically four species: *C. sabaeus*, *C. tantalus*, *C. aethiops*, and *C. pygerythrus* (Fig. 3.1). The analysis of Y-chr markers revealed a low genetic variability among the 30 samples with nine variable and seven parsimony-informative sites within the concatenated 1476 bp data set of ZFY and SRY. Accordingly, we refrained from reconstructing a rooted phylogenetic tree, and instead used median-joining networks to depict haplotype diversity and genetic distance among haplotypes and clades, respectively. We found six Y-chr haplotypes, whereas respective cyt *b* sequences represent 17 haplotypes forming six mtDNA clades. Y-chr sequences of *C. pygerythrus* from Southeast Ethiopia are identical to the *C. pygerythrus* sequence from Kenya/Tanzania and thus constitute a single haplotype, whereas mtDNA reveals two distinct clades, each for Southeast Ethiopia and Kenya/Tanzania (Fig. 3.2). Contrastingly, in concordance with mtDNA results *C. tantalus* samples from east of the Volta River and its confluents in Ghana and Burkina Faso reveal a distinct Y-chr haplotype, which differs from the haplotype obtained from East Nigerian *C. tantalus* samples. Y-chr sequences of *C. sabaeus* and *C. tantalus* from Ghana and Burkina Faso represent two different haplotypes, which are clearly separated east and west of the

Volta River reflecting the same pattern as mtDNA data (Fig. 3.2). The PCR based sexing method indicated that our data set did not include any sample of a male individual from West of the Volta River in South Ghana and consequently, only mtDNA information is available from this site (ID 203 in Fig. 3.1b).

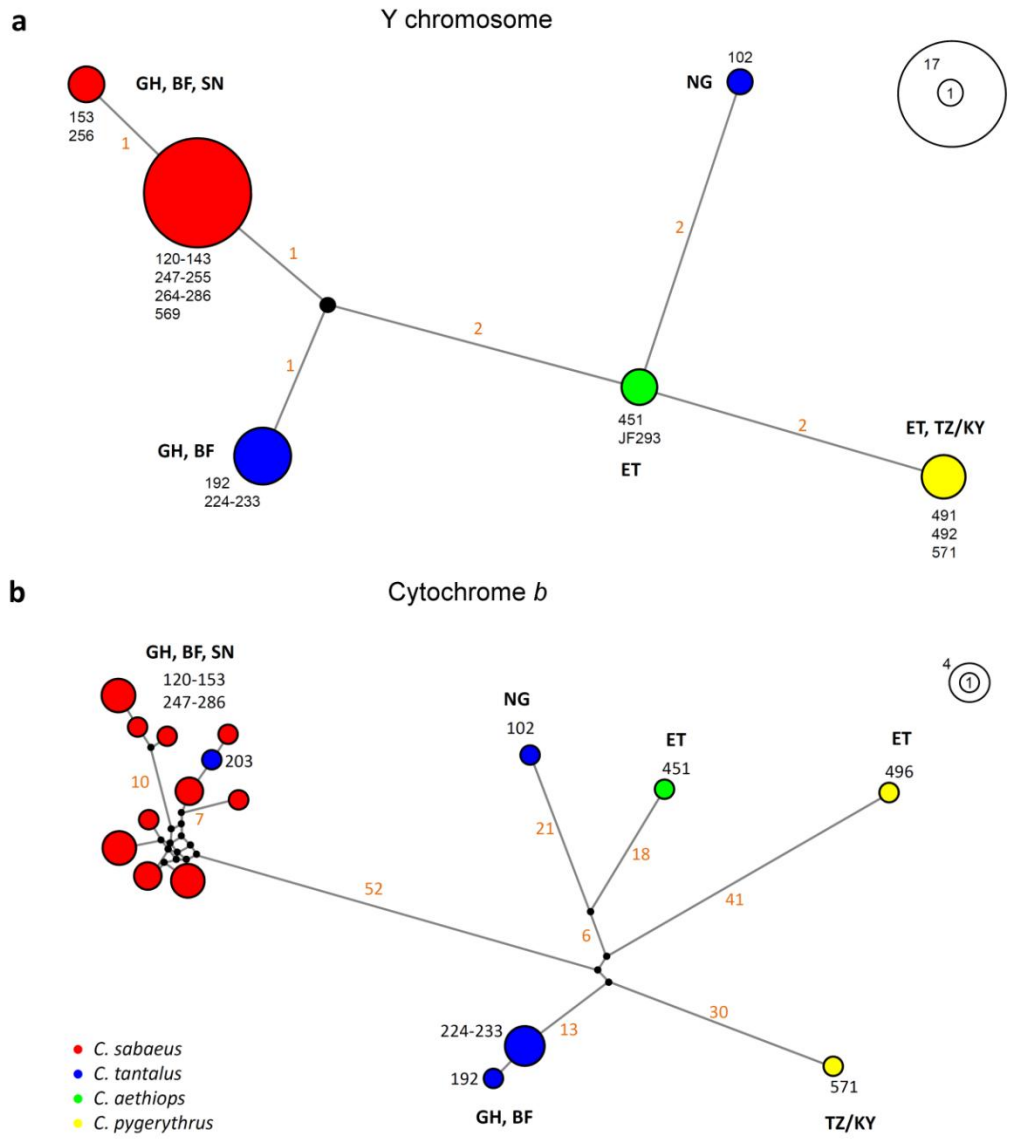

Fig. 3.2 Median-Joining networks of a) Y-chr and b) cytochrome *b* haplotypes. Circle sizes specify haplotype frequencies as shown by respective scales. Colours represent morphotypes, orange numbers indicate mutated positions of major branches, and black numbers correspond to sample IDs in Fig. 3.1b and Table 3.1. GH=Ghana, BF=Burkina Faso, SN=Senegal, NG=Nigeria, ET=Ethiopia, TZ/KY=Tanzania/Kenya.

DISCUSSION

Despite the morphological similarity, both Y-chr and mtDNA data support the distinctiveness of the western and the eastern *C. tantalus* clade (Fig. 3.1 and 3.2). Hence, introgressive hybridization is unlikely to have caused this paraphyletic pattern. Consequently, this discrepancy is possibly due to genetic variation assuming a new cryptic *Chlorocebus* taxon. So far, no clear morphological analysis has been conducted of specimens covering the whole range of *C. tantalus* and it remains unclear, if one of these clades is actually morphologically cryptic or if it probably represents slightly different morphological characters. Consequently, before we suggest any taxonomical changes, more precise morphological data as well as an analysis of further mitochondrial and nuclear loci are required. Nonetheless, Schwarz already reported a distinguishable morphotype from Togo in 1926 (Schwarz, 1926), but this form was not recognized by others (Booth, 1956; Hill, 1966). Moreover, grounded on some external characters that differ from *tantalus*, Blyth described the taxon *chrysurus* (Blyth, 1845), which is currently recognized as synonym of *tantalus* (Hill, 1966) or *sabaeus* (Napier, 1981; Groves, 2001). However, there is neither a type locality of *tantalus* nor of *chrysurus.* Consequently, if future analyses confirm our present results of the existence of two taxa, it needs to be clarified, which clade represents *tantalus* and which one a possible new taxon. Therefore, further genetic as well as morphological analyses should at best include type specimens of both *tantalus* and *chrysurus* to test whether these specimens belong to one of the detected genetic clades.

To examine if ancient introgressive hybridization is responsible for the disjunct mtDNA clades within East African *C. pygerythrus*, we analysed *C. pygerythrus* samples of the southeast Ethiopian mtDNA clade and of the mtDNA clade representing samples from Kenya and Tanzania (Fig. 3.1). In this case, both mtDNA clades revealed the same Y-chr haplotype. With respect to male mediated dispersal in African green monkeys (Kingdon, 1997; Jaffe & Isbell, 2011), ancient introgressive hybridization and nuclear swamping is the most probable explanation here, which led to a *C. pygerythrus* morphotype in Southeast Ethiopia carrying Y-chr DNA (and likely nuclear DNA) of *C. pygerythrus*, but mtDNA of a possible cytonuclear extinct taxon. Since we had no high quality male sample from South Africa, we were not able to analyse Y-chr DNA from the southernmost *C. pygerythrus* clade and it needs to be clarified, if in accordance with

morphology this mtDNA clade also represents the same (or a closely related) Y-chr haplotype as northern *C. pygerythrus* clades. Based on mtDNA the *C. pygerythrus* clade from Kenya and Tanzania forms a basal clade to the neighbouring *C. tantalus* and *C. aethiops* clades (Haus et al., submitted), and hybridization among *C. tantalus* and *C. pygerythrus* is still on-going in Uganda and Kenya (Fig. 1a; Napier, 1981; Kingdon, 1997). Similar to the above results, ancient introgression of proto-*C. tantalus*/*C. aethiops* mt genomes into *C. pygerythrus* populations by nuclear swamping is the most likely explanation for the paraphyletic relationships of *C. pygerythrus* within the mtDNA phylogeny.

The analysis of samples from the West African contact zone between *C. sabaeus* and *C. tantalus* revealed neither inconsistencies among genetic patterns of Y-chr and mtDNA, nor among Y-chr and morphological data excluding the possibility that hybridization occurred here. Consequently, there is no evidence of inter-specific gene flow among populations across the Volta River and its confluents in Northern Ghana and Burkina Faso. However, based on mtDNA introgressive hybridization has been found in a single *C. tantalus* population from the Accra plains west of the Volta River in South Ghana (Fig. 3.1a). This population carries mitochondrial genomes of *C. sabaeus*, although there is no evidence of morphological *C. sabaeus* individuals or groups in or close to this area (Haus et al., 2013). Unfortunately, samples from males of this population were not available, but according to above results we expect that this population carries Y-chr DNA of the neighbouring western *C. tantalus* clade due to ancient introgressive hybridization. Anyway, it remains unclear which factors have led to hybridization in the South, while there is no hybridization farther to the north despite less geographic barriers. Therefore we suggest that *C. tantalus* individuals from the eastern river bank were possibly introduced into a *C. sabaeus* population west of the Volta River by humans.

Our study highlights the importance of the analysis of Y-chr data in addition to mtDNA information to untangle evolutionary processes and resulting patterns of genetic diversity within animal taxa. Moreover, our results indicate that the evolution of African green monkeys was influenced by introgressive hybridization with the cytonuclear extinction of certain populations or taxa. Hybridization between *C. aethiops* and *C. djamdjamensis* in Ethiopia is still on-going and is assumed to play a threatening role

for the survival of the endemic species *C. djamdjamensis* from the South Ethiopian highlands (Kingdon, 1997; Mekonnen et al., 2012; Haus et al., 2013). Although natural induced hybridization is not unlikely in this case, it has been argued that deforestation and habitat disturbance most probably favour interspecific gene flow among these species (Kingdon, 1997). In accordance with our results, continuing asymmetric introgression can probably also lead to the cytonuclear extinction of the restricted species *C. djamdjamensis*. To analyse the progress and pattern of introgression within *C. djamdjamensis*, we recommend a comprehensive analysis of diverse mitochondrial and nuclear loci of samples including at best all remaining populations.

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CHAPTER 4

Geographical but not temporal concordance in the genetic structure of African savannah mammals

Tanja Haus[1,2,3], Michael Hofreiter[4], Christian Roos[2,5], Dietmar Zinner[1]

1 Cognitive Ethology Laboratory, German Primate Center, Leibniz Institute for Primate Research, Göttingen, Germany

2 Primate Genetics Laboratory, German Primate Center, Leibniz Institute for Primate Research, Göttingen, Germany

3 Centre of Biodiversity and Sustainable Land Use, University of Göttingen, Göttingen, Germany

4 Department of Biology, University of York, York, UK

5 Gene Bank of Primates, German Primate Center, Leibniz Institute for Primate Research, Göttingen, Germany

CR and DZ contributed equally

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ABSTRACT

Aim Diverse African savannah mammals reveal concordant phylogeographic patterns despite different ecological adaptations and habitat preferences. These patterns are commonly explained by the influence of Plio-Pleistocene glacial cycles and respective environmental shifts. Recent studies, however, suggest a more pronounced impact of regional climates on evolutionary processes particularly in East African species, including hominins. Using a set of two widely distributed primate and ungulate genera we intended to obtain more detailed information on trends and potential driving factors in the population history of African savannah mammals.

Location Savannah biome of the African continent.

Methods We used mitochondrial sequence information and fossil based calibration methods to date major phylogenetic and demographic events of African green monkeys (*Chlorocebus*), and applied similar methods to three co-distributed mammalian taxa: baboons (*Papio*), hartebeests (*Alcelaphus*) and warthogs (*Phacochoerus*).

Results Despite similar geographical distributions of major biogeographic clades, differences in divergence times and in population histories indicate less concordant phylogeographic processes among savannah mammals than previously assumed. Although some divergences coincide with humid phases in Africa, our estimates suggest that major splits also occurred during more arid periods. Furthermore, we found different patterns of population size variation between primate and ungulate genera and no clear temporal concordance of population size changes and of the Last Interglacial and Last Glacial Maximum of the Northern Hemisphere.

Main conclusions Major divergences within savannah mammals were likely induced by both extreme warm-humid and cold-arid climate periods and our results support the influence of more regional African climate variations than of climatic changes linked to Northern Hemisphere glacial cycles. Moreover, dissimilar patterns of population histories between primate and ungulate genera reflect taxon specific responses to environmental changes due to variant adaptations and habitat preferences. Our results suggest further comparative studies including savannah mammals of diverse guilds to obtain a more conclusive picture of the evolution of the African savannah biome.

Keywords: *Chlorocebus*, *Papio*, *Alcelaphus*, *Phacochoerus*, evolution, comparative phylogeography, Plio-Pleistocene climate, African climate variation

1. Introduction

Phylogenetic and population genetic studies on several sub-Saharan African savannah mammals revealed two different phylogeographic patterns. Whereas in some taxa a clear genetic separation of southern and northern lineages occurs with subsequent splits into eastern and western clades in the North (e.g., *Alcelaphus* (Arctander et al., 1999; Flagstad et al., 2001), *Giraffa camelopardalis* (Brown et al., 2007; Hassanin et al., 2007), *Papio* (Zinner et al., 2009b, 2013), *Panthera leo* (Bertola et al., 2011)), in other taxa an initial split between West and East African clades occurs in the North with subsequent divergence into a northeastern and a southern clade (e.g., *Phacochoerus africanus* (Muwanika et al., 2003), *Loxodonta africana* (Nyakaana et al., 2002), *Syncerus caffer* (Van Hooft et al., 2002), *Hippotragus equinus* (Alpers et al., 2004)). Furthermore, a comparison of population genetic data of African ungulates showed four major biogeographic areas in West, East, South, and Southwest Africa (Lorenzen et al., 2012). Inter-generic congruence of phylogeographic patterns is regarded as an indication of similar geological or climatic forces that affected the evolution of co-distributed species (Taberlet et al., 1998; Avise, 2009). Despite different ecological adaptations and habitat preferences of these mammalian taxa it has been assumed that climatic changes of the Pleistocene have similarly shaped their evolutionary history and population genetic structure (Arctander et al., 1999; Flagstad et al., 2001; Van Hooft et al., 2002; Alpers et al., 2004; Zinner et al., 2009b; Lorenzen et al., 2012). Accordingly, it has been suggested that irrespective of their geographical origin populations of savannah mammals were repressed into similar refugia in West, East, South and Southwest Africa during humid periods. Within these periods forests expanded and the savannah habitat retracted, which caused the genetic divergence of populations in these refugia leading to similar phylogeographic patterns in savannah taxa (Hassanin et al., 2007; Lorenzen et al., 2012).

Paleoclimatic and geological data as well as fossil records provide evidence that the increasingly dry climate at the end of the Pliocene (~2.8 mya [million years ago]) coincided with expanding grasslands in Africa, which in turn might have favoured the

expansion and diversification of arid-adapted mammals in general (Janis, 1993; DeMencoal, 1995; Vrba, 1999; deMenocal, 2004). However, recent studies propose a more complex picture. For instance, Trauth et al. (2005, 2007, 2009) suggested a more recent shift to increased climate variability in Africa, with significant humid periods superimposed on the globally increasing aridity, which might have affected speciation of hominins and other savannah mammals in East Africa. Beside this evidence of humid periods based on East African paleo-lake levels (Trauth et al., 2005, 2009), humid phases are also indicated in North Africa by increased discharge of the Nile River and the decrease of dust abundance in the East Mediterranean Sea (Larrasoana et al., 2003; Lourens et al., 2004).

Although phylogeographic studies on a number of savannah mammals have been conducted, only a minority considered divergence ages among different genetic lineages. Additionally, if divergence times have been included differing molecular dating methods were applied (Lorenzen et al., 2012), which hinders comparative phylogeographic approaches. Thus, a comparative molecular genetic analysis of intra-generic divergence time estimates, and relating those to major climatic shifts, can help obtaining a clearer picture on the impact of paleoclimatic factors on phylogeographic patterns of African savannah mammals.

Among ungulates and carnivores, several primate species occur in savannah habitats (Kingdon, 1997; Potts, 1998; Cerling et al., 2011). Whereas many of these savannah mammals have currently restricted distributions in Africa, either due to natural limits or caused by more recent anthropogenic influences, the distribution of African green monkeys (AGMs) of the genus *Chlorocebus* reflects almost the entire extension of savannah habitat throughout the continent (Hill, 1966; Kingdon, 1997); Fig. 4.1). This fact makes them a highly suitable model for a comparative phylogeographic approach.

In our study we aim to analyse the phylogeography, divergence times and main demographic changes throughout the evolution of the genus *Chlorocebus* using concatenated sequence information of the mitochondrial cytochrome *b* (cyt *b*) gene and the “Brown region”, which comprises the 3’end of the NADH dehydrogenase (ND) subunit IV gene (ND4), tRNA genes for histidine, serine, and leucine, and the 5’end of

the ND subunit V gene (ND5) (Brown et al., 1982). To investigate general phylogeographic trends for African savannah mammals and to evaluate the potential role of Plio-Pleistocene climatic fluctuations in their evolution, we applied similar methods to available sequence data of three other, widely distributed savannah mammals: baboons (Primates: *Papio*; Zinner et al., 2009b; Keller et al., 2010), common warthogs (Artiodactyla: *Phacochoerus africanus*; Muwanika et al., 2003) and hartebeests (Artiodactyla: *Alcelaphus*; Pitra et al., 1998; Arctander et al., 1999; Hassanin & Douzery, 1999; Matthee & Robinson, 1999; Flagstad et al., 2001). This allows us to infer general evolutionary trends in African savannah mammals as well as to detect potential differences in primates and ungulates.

METHODS

Data sampling

With the aim to include all six currently recognized AGM species (Groves, 2001, 2005; Haus et al., 2013) we collected 91 faecal samples at 32 sites covering a large part of the genus' range. However, as sample collection was limited due to time and budget we complemented this data set with museum material from different European and African museums representing the majority of the genus' distribution (Haus et al., 2013; Table S4.I). Faecal samples were preserved using the two-step method of Nsubuga et al. (Nsubuga et al., 2004) or in 90% ethanol, and were stored up to three years at -20°C until further processing.

Molecular methods

We used 126 complete mitochondrial cyt *b* gene (1140 bp) sequences of AGMs obtained from samples that we collected in several countries between 2005 and 2010 (Haus et al., 2013). To increase mitochondrial information for phylogenetic tree reconstructions in this study, we additionally amplified and sequenced the mitochondrial "Brown region" (Brown et al., 1982; Wildman et al., 2004; Zinner et al., 2009b) of 32 faecal samples representing the nine previously detected major cyt *b* clades (Haus et al., 2013; see the electronic supplementary material [ESM] for further details). All sequences were deposited in GenBank (Table S4.I).

Phylogenetic reconstructions

Based on a concatenated data set comprising the complete cyt *b* gene and the „Brown region“ of 32 samples we estimated phylogenetic relationships within AGMs. We built sequence alignments with Muscle implemented in MEGA 5.0 (Tamura et al., 2011) and checked them by eye. After adding an orthologous sequence of *Erythrocebus patas* as outgroup we removed indels with the software Gblocks 0.91 (Castresana, 2000; Talavera & Castresana, 2007) and analysed best-fit models of nucleotide substitutions for each locus (cyt *b*, ND4, tRNAs, and ND5), separately, based on the Bayesian Information Criterion (BIC) using the program jModeltest 0.1 (Guindon & Gascuel, 2003; Posada, 2008). To reconstruct phylogenetic relationships we applied Maximum Likelihood (ML) and Bayesian approaches as implemented in Garli 2.0 (Zwickl, 2006) and MrBayes 3.1.2 (Huelsenbeck et al., 2001; Ronquist & Huelsenbeck, 2003). We adjusted model specification settings according to jModeltest results of data partitions, while all other settings were left at their default values. We ran four independent ML analyses with 500 bootstrap steps each and reconstructed a 50% majority rule consensus tree in Paup* 4b10 (Swofford, 2003). For the Bayesian approach we did three independent runs with 10,000,000 generations and tree and parameter sampling every 1,000 generations. Average standard frequencies were below 0.01 after 10,000,000 generations and the uncorrected Potential Scale Reduction Factor (PSRF) approached one. The first twenty-five percent of sampled trees and parameters were discarded as burn-in. Final trees were viewed and edited using FigTree 1.3.1.

Selection of comparative data sets

For the comparative approach we selected savannah mammal genera based on three main criteria: 1) they should exhibit similar geographic distributions in the African savannah biome; 2) genera should be represented by good sampling coverage; and 3) appropriate genetic data should be available. Based on these preconditions we included data of one other primate genus, baboons comprising 68 samples (Zinner et al., 2009b; Keller et al., 2010), and two ungulate genera, hartebeests (Pitra et al., 1998; Arctander et al., 1999; Hassanin & Douzery, 1999; Matthee & Robinson, 1999; Flagstad et al., 2001), and common warthog (Muwanika et al., 2003) comprising 147 and 70 samples, respectively (Fig. 4.1, Table S4.I).

To fill some gaps in the baboon data set we additionally included 17 faecal samples from previously not sampled geographical regions. The complete cyt *b* gene and the "Brown region" of these additional baboon samples were amplified and sequenced according to Zinner et al. (2009b) and Keller et al. (2010). The complete data set of all four genera comprises 428 sequences (Table S4.I, Fig. 4.1).

Divergence time estimates

Selection of genetic loci for each genus was based on the availability and quality of sequence data from GenBank. This way final data sets for the comparative phylogenetic analysis of divergence times constituted 32 AGM (2034 bp) and 18 baboon (2032 bp; (Zinner et al., 2009b) concatenated cyt *b* and "Brown region" sequences, 30 common warthog D-loop (341 bp; Muwanika et al., 2003), and 15 hartebeest partial cyt *b* (417 bp; Pitra et al., 1998; Arctander et al., 1999; Hassanin & Douzery, 1999; Matthee & Robinson, 1999; Flagstad et al., 2001) sequences. To analyse divergence times we used Bayesian algorithms as implemented in BEAST 1.6.2 (Drummond & Rambaut, 2007). We calibrated molecular clocks consistently with direct fossil dates or fossil based time estimates derived from molecular studies to analyse divergence times (see the ESM for further details). Models of nucleotide substitutions were set according to jModeltest results. We furthermore partitioned coding genes into two partitions (coding positions 1+2 and 3, respectively) and used relaxed clock models with uncorrelated lognormal rates for all data sets assuming a birth death process for the tree prior (Drummond et al., 2006).

Demographic reconstructions

To obtain information on more recent evolutionary processes within genera, we analysed demographic changes through time. For the loci that we used in the divergence time analysis not enough data are available to conduct separate analyses of biogeographic clades, except for the D-loop sequences of the common warthog. Therefore, we chose the complete cyt *b* gene for primate genera and a partial D-loop sequence for ungulate taxa, as for these loci large data sets are available from GenBank. To estimate demographic changes through time we used 126 cyt *b* sequences of AGMs

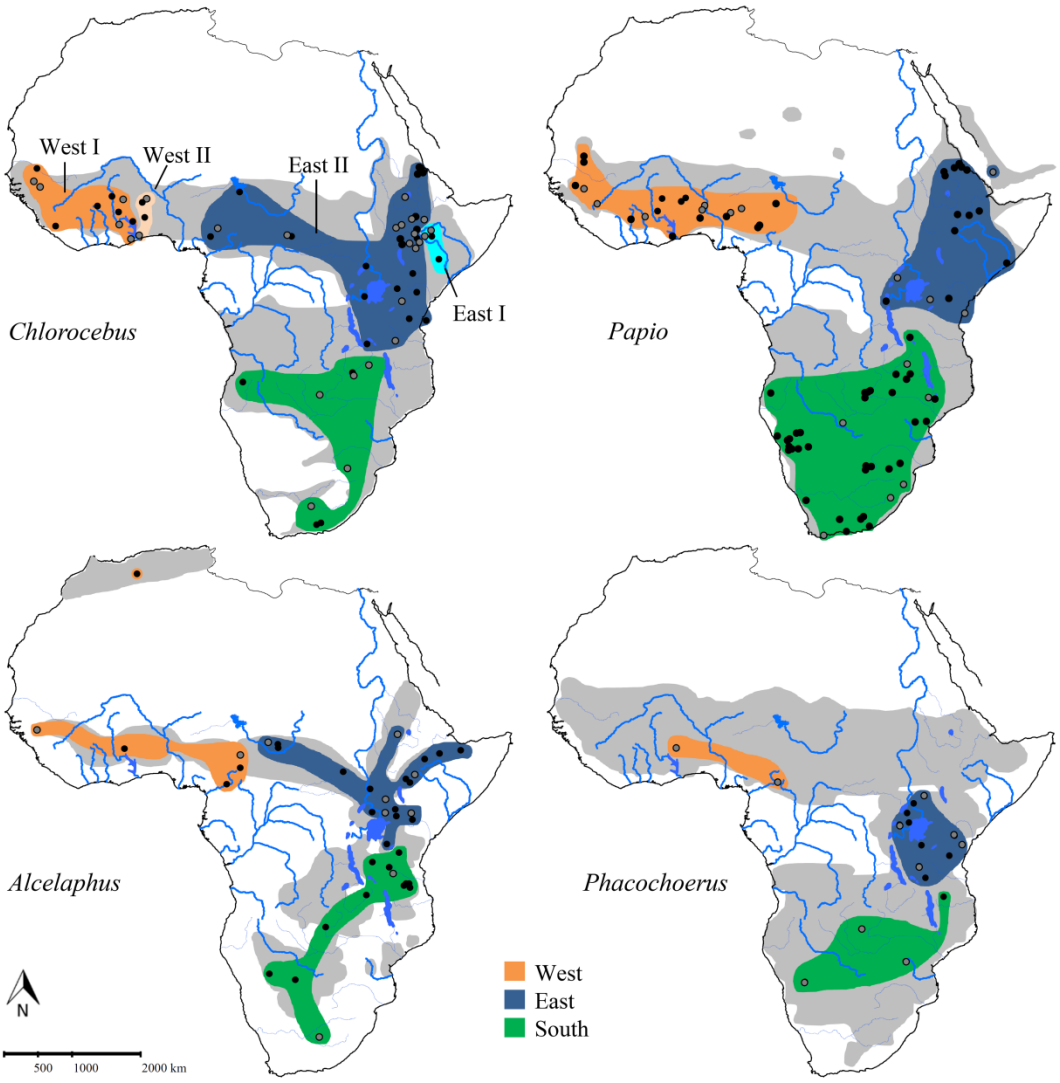

Fig. 4.1 Distribution, sample sites and biogeographic clades of the investigated taxa. Current distributions are indicated by grey shaded areas (Kingdon, 1997). Grey dots indicate samples used for phylogenetic reconstructions; black dots indicate additional samples used for demographic analyses. Detailed information on sample origins and sequences are listed in Table S4.1.

and 85 of baboons (1140 bp), and 142 and 70 D-loop sequences of hartebeests (316 bp) and common warthogs (369 bp), respectively. We estimated Bayesian Skyline Plots (BSP) of each biogeographic clade (West, East, and South) separately using the software BEAST (Drummond et al., 2005; Drummond & Rambaut, 2007). We applied relaxed clock models with uncorrelated lognormal rates to accommodate rate variation among lineages (Drummond et al., 2006) and completed three independent runs of

100,000,000 generations with tree and parameter sampling every 10,000 generations. For the calibration of time scales we used mean estimates of mitochondrial substitution rates obtained from our previous divergence time estimations, except for *Alcelaphus*, for which we used substitution rates of the mitochondrial D-loop of bisons (Shapiro et al., 2004). Detailed methods on BSP reconstructions are found in the ESM.

RESULTS AND DISCUSSION

Phylogeography of African green monkeys

ML analyses of the concatenated "Brown region" and cyt *b* data set of AGMs revealed identical topologies for all four independent runs (log likelihoods -6980.9595 to -6980.9598) with largely similar topologies supported by the Bayesian approach (Fig. S4.1). We obtained nine well supported mitochondrial DNA clades based on both ML and Bayesian phylogenetic reconstructions, which separated from each other more than 1 mya (Figs. S4.1, 4.2a). These clades were also recovered in an earlier comprehensive cyt *b* phylogeny (Haus et al., 2013). However, in contrast to Haus et al. (2013) we found stronger supported phylogenetic relationships (Figs. 4.2a, S4.1). The phylogeny of the genus reflects a complex picture with independent secondary diversifications in East and West Africa. The initial split within AGMs occurred around 2.46 mya (95% highest posterior density [HPD] interval: 1.89-3.09 mya) in the early Pleistocene, separating a western clade (West I) from the remaining lineages of the genus. The age of this first split is supported by previous estimates of 2.81 (±0.35) and 2.76 (±0.23) mya deriving from a co-evolutionary study in AGMs and their specific Simian immunodeficiency viruses (SIV) (Wertheim & Worobey, 2007). The second major event shows the divergence of an eastern (East I) clade, occurring in Ethiopia and Somalia, from all remaining clades (Figs. S4.1, 4.2). Subsequent major splits occurred within a short time period (1.56-1.15 mya) and included a second western clade (West II) from Ghana and Burkina Faso, a South African clade, as well as the divergence of a second eastern clade (East II, Fig. 4.2). Rapid diversifications are also known from other savannah mammals (Matthee & Davis, 2001; Hassanin & Ropiquet, 2004; Zinner et al., 2009b, 2013), which has been regarded as response to climatic changes.

Based on morphological data, origins of AGMs were considered to be either in South or East Africa (Schwarz, 1926; Hill, 1966; Kingdon, 1984). However, according to our findings a West African origin of the genus, with a subsequent immigration into Eastern and Southern Africa, is most likely. This is also in agreement with results of the co-evolutionary study (Wertheim & Worobey, 2007) and with the fossil record (Elton, 2007). Assuming that central African rain forests constituted a barrier to the dispersal of AGMs, there are two possible routes for the migration into Southern Africa: along a savannah corridor in East Africa, or alternatively, through a savannah corridor further west between Central African forest refugia during periods of dry climate (Kingdon, 1984; Hamilton & Taylor, 1991; Nichol, 1999). Interestingly, both dispersal routes, although in opposite direction from south to north, have also been proposed for baboons (Zinner et al., 2011b).

Phylogeographic pattern and timing of the evolution of African savannah mammals

Our analyses revealed no differences in phylogenetic relationships compared to previous molecular phylogenies of baboons, hartebeests and common warthogs. This was expected since we used almost identical data sets as in earlier studies of these taxa (Arctander et al., 1999; Flagstad et al., 2001; Muwanika et al., 2003; Zinner et al., 2009b; Keller et al., 2010). To compare phylogeographic patterns we defined three major biogeographic areas: West, East and South Africa and focussed on the respective clades. These three biogeographic groups are typically found in African ungulates (Lorenzen et al., 2012) and other savannah mammals, e.g., baboons and lions (Zinner et al., 2009b; Bertola et al., 2011). Phylogenetic relationships within AGMs, however, indicate a more complex evolutionary history than those of baboons, hartebeests or warthogs. In AGMs samples from the three geographical areas do not represent monophyletic clades. Two distinct lineages are found in East and in West Africa, respectively (Figs. 4.1 and 4.2). In addition, a comparison of the phylogenies of the four mammalian genera reveals some fundamental inter-generic differences in the relationships among clades. Baboons and hartebeests show initial divergences between northern and southern clades (Arctander et al., 1999; Flagstad et al., 2001; Zinner et al., 2009b, 2013), whereas the initial split in AGMs occurred between the West African clade I and the remaining lineages, similar to the pattern found in common warthogs (Muwanika et al., 2003). Furthermore, in

baboons and hartebeests, the eastern clade constitutes a sister group to the western clade (Arctander et al., 1999; Flagstad et al., 2001; Zinner et al., 2009b), whereas in common warthogs, the eastern clade constitutes a sister group to the southern clade, suggesting that the east was colonized from southern regions (Muwanika et al., 2003; Lorenzen et al., 2012). The phylogenetic placement of the East I clade of AGMs remains uncertain, but the position of East II in the phylogeny indicates a possible second colonization of eastern Africa also from southern regions.

Divergence time estimations revealed an initial diversification of AGMs and baboons in the early Pleistocene at 2.46 (1.89-3.09) mya and 2.16 (1.79-2.58) mya, respectively, whereas ungulates started radiating earlier in the Pliocene, with hartebeests at 3.71 (2.26-5.53) mya and common warthogs at 4.57 (2.86-6.46) mya (Fig. 4.2). A previous study on hartebeests found a considerably younger time estimate for the initial North-South split of this taxon in the late Pleistocene (Flagstad et al., 2001). An explanation for this discrepancy may be that we calibrated our molecular dates with calculated means of more recently published data on divergence times of Antilopinae, Aepycerotini-Neotragini, and Hippotragini-Alcelaphini (Hassanin & Ropiquet, 2004; Ropiquet & Hassanin, 2005; Hassanin et al., 2012); see ESM), which were not available for the earlier study. Based on these calibration points we estimated the divergence age between *Connochaetes gnou* and *C. taurinus* at c. 2.05 mya (Fig. 4.2c), which conforms to previous estimates based on fossil and genetic data ranging from 2.0-2.5 mya (Vrba, 1979; Fernández & Vrba, 2005). Furthermore, Fernandez & Vrba (2005) provided a divergence age of the tribe Alcelaphini at 10±1.3 mya, which is also in concordance with our age estimate at 9.41 (6.46-12.59) mya (Fig. 4.2c). In ungulates time estimates indicate broad HPD intervals, which can be explained by the large standard deviations of mean calibration points (see ESM). Inferences on temporal concordance of evolutionary and climatic events must therefore be treated with caution. However, based on median estimates we found that splits separating northern and southern clades occurred at c. 1.44 mya in AGMs, 2.16 mya in baboons, and 3.71 mya in hartebeests, while splits among western and eastern clades occurred at c. 2.46 mya, 1.94 mya, and 2.47 mya, respectively. Due to the closer relationship of the eastern and southern clades in common warthogs, the north-south split occurred here at c. 4.57 mya and the south-

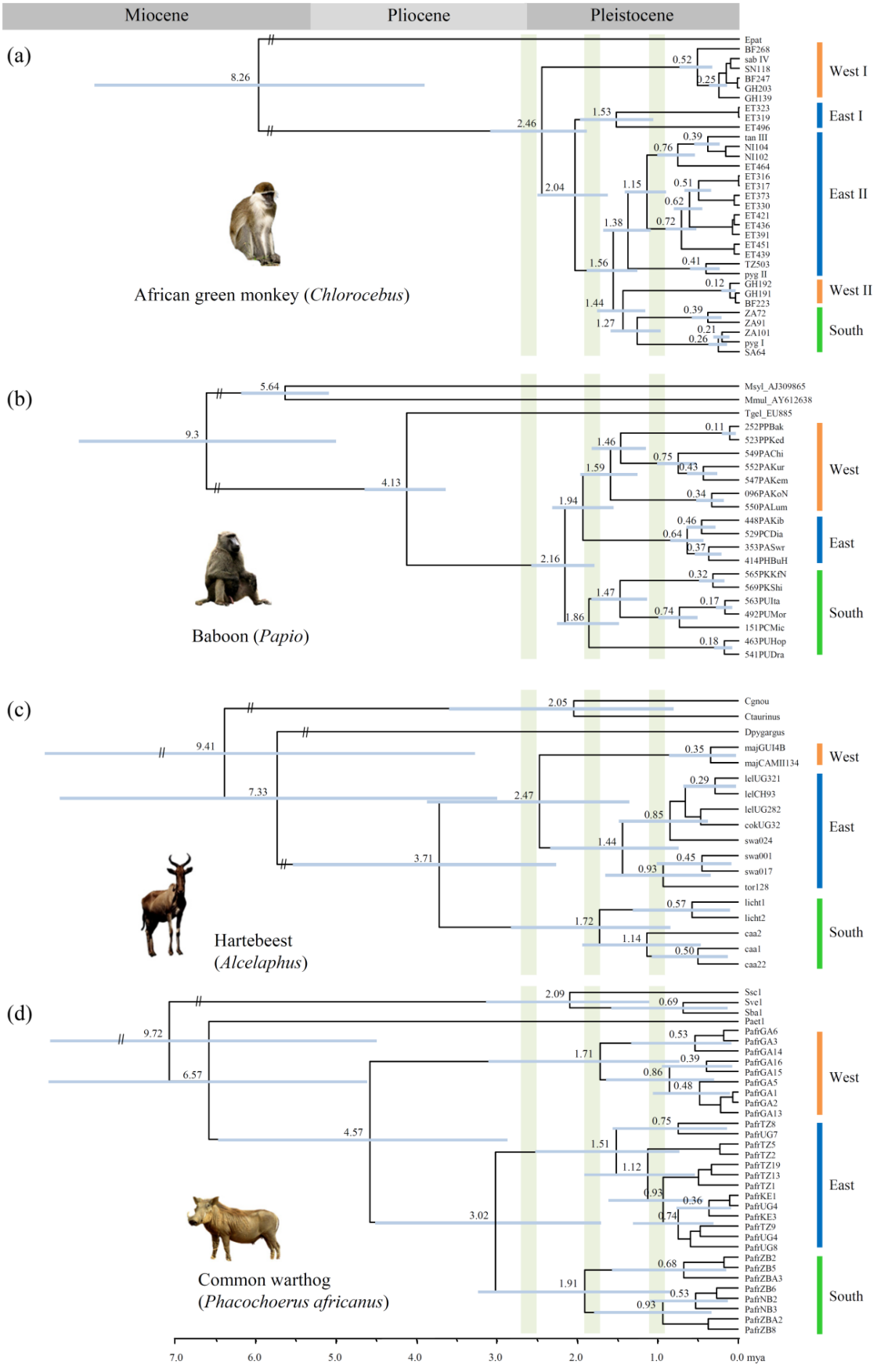
Miocene
Pliocene
Pleistocene
(a)
African green monkey (Chlorocebus)
(b)
Baboon (Papio)
(c)
Hartebeest (Alcelaphus)
(d)
Common warthog (Phacochoerus africanus)
West I
East I
East II
West II
South
West
East
South
West
East
South
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0.0 mya

Fig. 4.2

Fig. 4.2 Phylogeny of *Chlorocebus* in comparison to other African savannah mammals and major climate changes. Phylogenetic reconstructions, major biogeographic clades, and divergence time estimates (numbers above nodes) of (a) *Chlorocebus*, (b) *Papio*, (c) *Alcelaphus*, and (d) *Phacochoerus*. Phylogenetic reconstructions are shown back to c. 9 mya. Green shadowed intervals indicate shifts to high moisture levels (Trauth et al., 2005, 2007).

east split around 3.02 mya (Fig. 4.2). Consequently, there is no obvious overall concordance of divergence ages among the four mammal genera, suggesting independent evolutionary processes in these taxa. Furthermore, according to Trauth et al. (2007) significant humid periods prevailed in Central and Northern Africa at 2.7-2.5 mya, 1.9-1.7 mya, and 1.1-0.9 mya, which superposed the general trend of increasing aridity (Fig. 4.2). However, our median divergence ages indicate neither clear coincidence, nor general discordance with those humid periods, even though major splits likely occurred predominantly during drier time periods (Fig. 4.2). However, due to the application of different calibration points and respective substitution rates to phylogenies of considered genera, our results might depict a wrong picture. For example, higher substitution rates within ungulate genera would probably produce a more concordant temporal pattern among major ungulate splits and humid periods. Nevertheless, based on depicted phylogenies of primate genera we do not expect concordant patterns among each other or compared to ungulate genera and humid periods irrespective of substitution rates. Therefore, major splits of savannah mammals most likely occurred during both more arid conditions as well as during humid periods. This contradicts the previous hypothesis that divergences of savannah mammals occurred in periods of humid phases when the savannah biome was divided into several refugia (Hassanin et al., 2007; Lorenzen et al., 2012). In contrast to typical rain forest and desert habitats, which show either extension or retraction in response to climatic changes, the extension of the savannah biome might be impacted by both droughts and wet periods. Either condition would lead to a retraction of savannah areas with subsequent separation of savannah mammal populations during times of climate extremes. Under such a scenario an extension of the savannah biome with an accompanying expansion of savannah adapted mammals would be expected during intermediate climate periods.

Despite the inter-generic differences in phylogenetic relationships and divergence times, current distributions of biogeographic clades of the four mammal taxa show remarkable overlapping geographic ranges (Fig. 4.1). Even though there are sampling gaps for baboons and common warthogs in Central or Northeast Africa, in general, western clades seem to expand into the east at least up to East Nigeria and Cameroon (baboons, hartebeests, and common warthogs), and ranges of East African clades stretch as far as Chad and East Nigeria in the West (AGMs and hartebeests). An even more concordant pattern is found for the east-south border, which is located in the southern East African Rift System (EARS) as all eastern clades expand south up to Tanzania, and southern clades up north to Tanzania or at least Zimbabwe (Fig. 4.1). Similar patterns are also found in other savannah mammals and arid adapted plant taxa (Juergens, 1997; Zinner et al., 2011b; Lorenzen et al., 2012 and therein). Different factors might have led to these concordant distributions. In Central Africa both the southern extension of the Saharan desert up to one or two degrees of the Equator during extreme droughts (Nichol, 1999) and the Lake Mega-Chad and surrounding drainage systems during maxima of humidity might have constituted barriers to the dispersal of populations in West and East Africa (Goudie, 2005; Hassanin et al., 2007; Zinner et al., 2011b). In Southeast Africa also different factors likely explain concordant distributions. During humid phases, eastern South Africa and southern Mozambique were dominated by evergreen and deciduous forests and the equatorial forest extended to the coast (Cowling et al., 2008; Dupont et al., 2011). However, also extreme droughts have been proposed based on paleoecological records from Lake Malawi in tropical Africa for the late Pleistocene (Scholz et al., 2007). Moreover, the EARS environment with its numerous lakes and dry valleys has been repeatedly influenced by climatic and also tectonic events during the Plio-Pleistocene with significant humid periods and alternating arid conditions as well as recurring volcanism (Trauth et al., 2007, 2009, 2010; Basell, 2008). These environmental fluctuations might have repeatedly reduced the possibility of north-south dispersal through a savannah corridor in East Africa and in turn have induced a separation of northern and southern African savannah populations.

Late Pleistocene and Holocene demographic changes

To analyse more recent evolutionary processes we conducted coalescent based demographic analyses, estimating changes in effective population sizes through time, of western, eastern and southern biogeographic clades separately. Changes in population sizes do not show consistent patterns among biogeographic clades of different genera, although patterns are more similar within primates than within ungulates (Fig. 4.3). For instance, BSPs indicate a decline or increase, of population sizes in western clades, an increase with following decline in eastern primate clades, but population expansion in ungulate clades. Whereas in primate genera population declines seem to start after the last interglacial period (LIG: 110-126 kya; Tzedakis, 2003) or during the last glacial maximum (LGM: 22-19 kya; Johnsen et al., 1992; Yokoyama et al., 2000), population expansions, starting around 200 kya or 50 kya, do not coincide with the LIG or the LGM (Fig. 4.3a-b). In ungulate genera, population sizes increased around 200 kya and 100 kya with further sudden population expansions at 12-10 kya and c. 15 kya (Fig. 4.3c), or population sizes increased constantly within last 500 kya (Fig. 4.3d). Therefore, also in ungulate genera no consistent relation between LIG and LGM and population size changes can be detected. Low-latitude warming and monsoon dynamics are major drivers of the hydrological cycle in tropical Africa, and local differences in the East African climate are known to have superimposed global climate transitions (Trauth et al., 2005, 2010). Therefore, our results might reflect that climatic changes during the LIG and LGM of the Northern Hemisphere might have been of less importance for recent demographic changes in African savannah mammals. However, it has been shown that analyses of multiple independent loci of small sample sizes resolve demographic changes back in time much better than a single locus of large data sets (Heled & Drummond, 2008). Since we used only mitochondrial cyt *b* gene or D-loop sequences, our data possibly do not provide enough genetic information to completely resolve demographic patterns.

Irrespective of the timing of population size changes our results indicate that despite similar geographic distributions, different savannah mammals show different demographic patterns during the late Pleistocene and Holocene (Fig. 4.3). The African savannah represents and always has represented a heterogeneous ecosystem and there

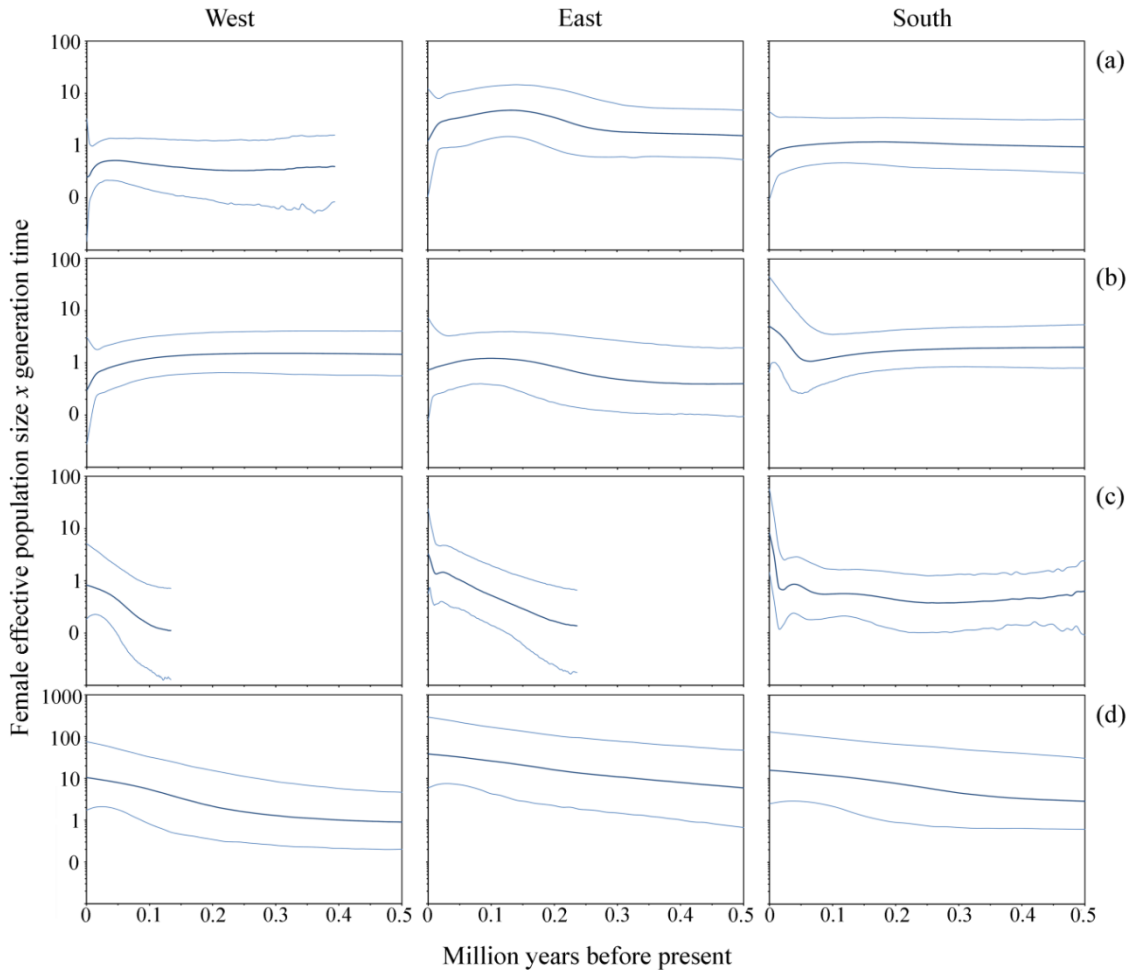

Fig. 4.3 Bayesian Skyline Plots (BSP) showing median changes (thick lines) of female effective population size (N_{ef}) multiplied by generation times (τ) with upper and lower highest posterior density intervals in millions through time. Analyses were conducted separately for biogeographic clades of (a) *Chlorocebus* (cyt *b*), (b) *Papio* (cyt *b*), (c) *Alcelaphus* (D-loop), and (d) *Phacochoerus africanus* (D-loop). Due to small sample sizes in East I and West II clades of *Chlorocebus*, only demographic changes of West I, East II and South are depicted here (see Fig. 4.2).

are different types of savannah based on moisture and nutrient levels as well as on the tree/grass ratio, which offers a broad range of different habitats (Belsky, 1990; Toit & Cumming, 1999). The primate genera considered here are mainly frugivorous and are adapted to open savannah habitats and a more terrestrial life style compared to most forest adapted primates (Hill, 1966). Nevertheless, their occurrence still depends on the availability of fruiting trees as main food resource, especially in AGMs. The two ungulate genera are predominantly grazers, live exclusively terrestrial (Kingdon, 1997) and trees are not a necessary prerequisite for their occurrence. These ecological differences

certainly have influenced patterns and timing of demographic events in the different species.

CONCLUSIONS

Our comparison of AGMs with three other co-distributed African savannah mammals reveals a less consistent phylogeographic pattern than previously assumed, which suggests independent evolutionary processes for each genus. Irrespective of the geographic and temporal origin of the genus, pronounced environmental shifts in Central and East Africa possibly led to concordant general biogeographic patterns. We assume that savannah refugia existed during both periods of extreme wetness and drought, which might have led to the interference of diverse climatic conditions involved in the evolution of African savannah mammals. Based on recent demographic changes we assume that low-latitude regional climatic forces influenced evolutionary histories of African savannah mammals more than global climatic changes related to the LIG and the LGM. Accordingly, our results support the hypothesis on the influence of regional climatic forces on speciation processes in Africa, which likely have played an important role in the evolution of hominins (Trauth et al., 2010). Here, the inclusion of additional mammalian savannah taxa of different guilds in future comparative phylogeographic approaches will provide more detailed information on local climatic and environmental changes and a more conclusive picture of the evolution of the savannah biome. Due to the broad confidence intervals of our time estimates we suggest future studies on comprehensive data sets considering both complete mitochondrial genomes and nuclear DNA information in order to obtain more precise estimates of the timing of evolutionary events in these species.

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SUPPLEMENTARY MATERIAL

1. Supplementary material Methods

Molecular methods

We amplified and sequenced the mitochondrial "Brown region" (Brown et al., 1982; Wildman et al., 2004; Zinner et al., 2009b) from 32 faecal samples which derived from individuals that represent the nine previously detected major cytochrome *b* (cyt *b*) clades of African green monkeys (Haus et al., 2013). We extracted DNA from faecal samples using the Qiagen DNA Stool Mini Kit (Qiagen, Germany). The complete "Brown region", which comprises 897 bp in African green monkeys (3'end of the NADH dehydrogenase (ND) subunit IV gene (ND4): 457 bp; tRNA genes for histidine, serine, and leucine: 201 bp; 5'end of the ND subunit V gene (ND5): 239 bp), was amplified by two overlapping fragments using primers BR1fw GTAAGCCATATATCCCTAGTAA and BR1rv TCTTATTTACCGAGAAAACTCG, and BR2fw CTCCTGTAAATATAGTTTAACCA and BR2rv CAATCGCACTATTCATCACCT, respectively. We used a 30 µl PCR mix (1 U BiothermTaq 5000 (Genecraft, Germany), 1x reaction buffer, 0.16 mM for each dNTP, 0.33 µM for each primer, and 0.6 mg/ml BSA, and set thermo cycler conditions to 94°C for 2 min, followed by 40 cycles of 94°C for 1 min, 60°C for 1 min, 72°C for 1 min, and 72°C for 5 min. We ran all polymerase chain reaction (PCR) products including PCR blanks on 1-2% agarose gels. After excision, PCR products were purified with the Qiagen Gel

Extraction Kit (Qiagen, Germany). Sequences were run on an ABI 3130*xL* sequencer with respective forward and reverse primers using the BigDye Terminator Cycle Sequencing Kit (Applied Biosystems, Germany).

Divergence time estimations

For each genus we used five to nine outgroup orthologs for rooting and calibration purposes (Table S4.I). All data sets were aligned with Muscle implemented in the program MEGA 5.0 (Tamura et al., 2011). Indels and ambiguously aligned blocks were removed using the program G-blocks 0.91 (Castresana, 2000; Talavera & Castresana, 2007). We estimated best-fit models of nucleotide substitutions based on the BIC using jModeltest 0.1 (Guindon & Gascuel, 2003; Posada, 2008). For the concatenated data sets of cyt *b* and "Brown region", models of nucleotide substitutions were estimated separately for each locus (cyt *b*, ND4, tRNA and ND5).

To calibrate phylogenies of savannah mammals we used direct fossil calibration points or fossil based times estimates derived from molecular studies and described them by using normal distributions with given mean and standard deviations (SD) using the software BEAST 1.6.2 (Drummond & Rambaut, 2007). As pointed out in Lorenzen et al. (2012), there are problems in estimating intraspecific divergence times based on fossil calibrations (Ho et al., 2008). However, as all studied taxa show strong genetic sequence divergences among main biogeographic clades, we argue that this fact is less important in our study. For African green monkeys and baboons we calibrated splits between Cercopithecoidea and Hominoidea (26.5 ±2.5 mya; Zalmout et al., 2010; Pozzi et al., 2011), *Homo* and *Pan* (6.5 ±0.5 mya; Vignaud et al., 2002; Brunet et al., 2005; Lebatard et al., 2008), *Macaca mulatta* and *M. sylvanus* (5.5 ±0.5 mya; Delson, 1980), and between *Theropithecus* and *Papio* (4.0 ±0.5 mya; Leakey, 1993; Delson, 2000). To calibrate the hartebeest phylogeny we calculated means and SDs of previously published results to include uncertainties of time estimates as indicated by large differences between studies (Hassanin & Ropiquet, 2004; Ropiquet & Hassanin, 2005; Hassanin et al., 2012). This resulted in following means and SDs (±SD): 16.62 ±1.56 mya for the divergence of Antilopinae, 15.54 ±1.67 mya of Aepycerotini and Neotragini, and 12.95 ±1.72 mya for the divergence of Alcelaphini and Hippotragini. Further, we used

information on four nodes to calibrate the warthog phylogeny (Gongora et al., 2011): the split between *Babyrousa* and *Sus*/*Phacochoerus* (13.25 ±2.15 mya), *Sus* and *Phacochoerus* (10.84 ±1.8 mya), the initial divergence within *Sus* (2.49 ±0.6 mya), and the divergence of *Phacochoerus aethiopicus* and *P. africanus* (5.74 ±1.5 mya). We completed three Markov chain Monte Carlo (MCMC) runs with 10,000,000 generations sampled every 1,000 generations. An examination of the log-file with Tracer 1.5 (Rambaut & Drummond, 2007) showed that effective sample sizes (ESS) of all parameters were more than 200 and converged to the posterior distribution representing an adequate sample. We deleted 25% as burn-in and combined resulting tree samples of each of the three runs with LogCombiner 1.6.2. Finally, trees were summarized and visualized with TreeAnnotator 1.6.2 and FigTree 1.3.1.

Demographic reconstructions

We divided the data sets into Western, Eastern, and Southern groups according to major geographic clades (Fig. 4.2). Since we had only few data in the East I and West II clade of African green monkeys we considered only West I, East II and the Southern clade for the demographic analysis. Although phylogenetic relationships of all data sets, except of some baboon samples, were analysed previously, we reconstructed Neighbor-Joining trees with MEGA 5.0 (Tamura et al., 2011) to ensure accurate assignment of samples to respective geographic groups. Using a MCMC sampling algorithm we estimated changes through time with a linear approach. Best-fit models of nucleotide substitutions were estimated for each data set and geographic group separately based on the BIC as implemented in jModeltest 0.1 (Guindon & Gascuel, 2003; Posada, 2008). We additionally chose a partition into codon positions (coding positions 1+2 and 3, respectively) for the coding cyt *b* gene. We applied a relaxed Bayesian clock model with uncorrelated lognormal rates to accommodate rate variations among lineages (Drummond et al., 2006). We tested the adequacy of different amounts of chain points starting with 5 groups for sample sets of less than 50 samples, and with 10 groups for sets of more than 50 samples. We then increased group numbers carefully until ESS values and the 95% credibility intervals reached inadequate values (not shown here). We calibrated the time scale using mean estimates of mitochondrial substitution rates of previously conducted BEAST runs (see above): 1.80×10^{-2} (African green monkeys), 1.73

x 10^{-2} (baboons), 1.01 x 10^{-2} (common warthogs) substitutions per site per million years. Due to differences in mutation rates of the cyt *b* gene and the partial D-loop sequence we used an evolutionary rate estimate of 32% per million years for hartebeests, which was estimated for the bison mitochondrial control region (Shapiro et al., 2004). We completed three independent runs of 100,000,000 generations with tree and parameter sampling every 10,000 generations. Visual inspections of log-files in Tracer 1.5 (Rambaut & Drummond, 2007) indicated that all ESS values were more than 200, and traces showed convergence of posterior distributions for all data sets and parameters. Twenty-five per cent of first trees and parameters were discarded as burn-in and log and tree files of the three separate runs were combined for BSP reconstructions using LogCombiner. BSPs were finally reconstructed and edited with Tracer 1.5 (Rambaut & Drummond, 2007) and Excel.

2. Supplementary material Figure

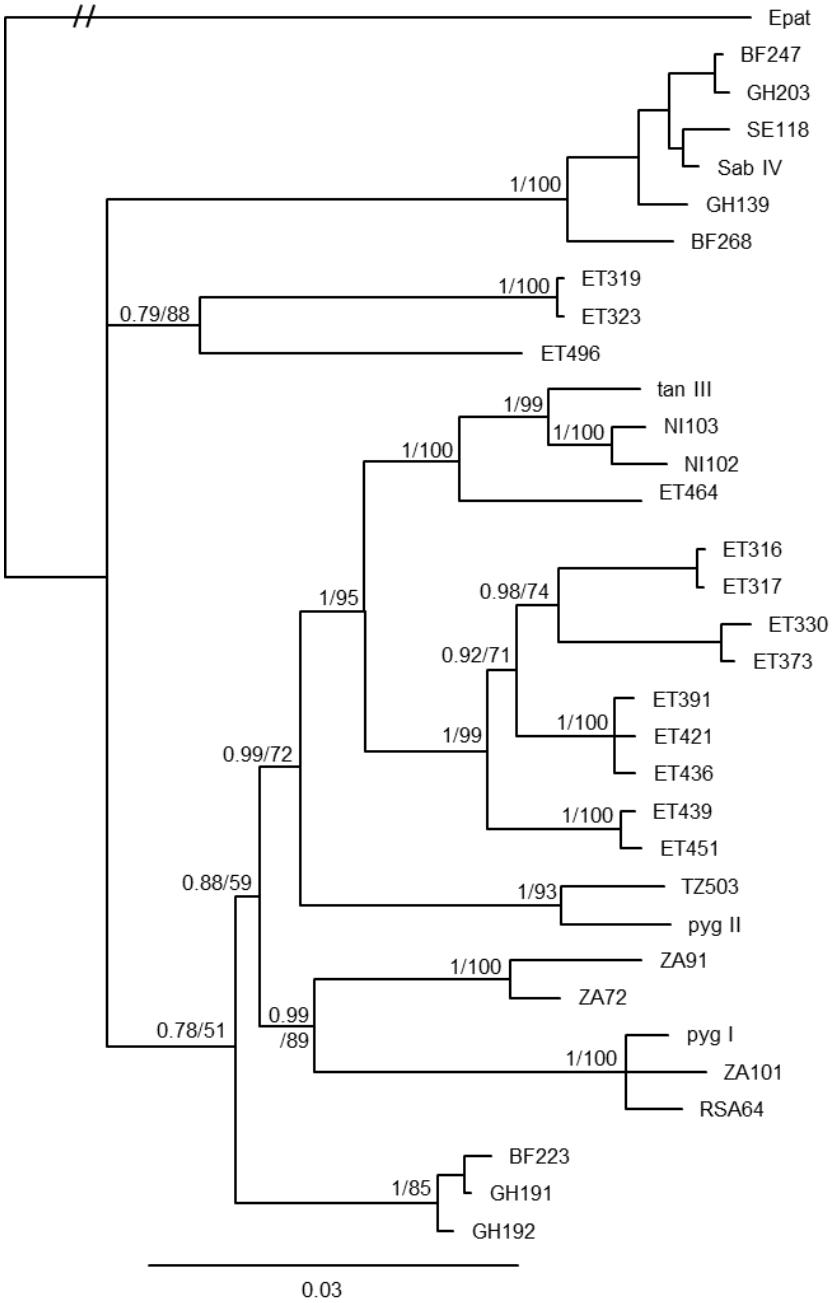

Fig. S4.1 Phylogenetic reconstructions of African green monkeys (*Chlorocebus*), showing posterior probabilities and bootstrap supports of Bayesian and Maximum Likelihood approaches at respective nodes.

3. Supplementary material Table S4.I

See appendix I in the end of this thesis**.**

CHAPTER 5 General Discussion

In the general discussion I will revisit major findings of my thesis with the aim to obtain a conclusive picture on the evolutionary history of African green monkeys and the possible impact of hybridization on their phylogeny. I then compare my data with other African savannah mammals to infer major evolutionary trends in the evolution of these African groups during the last million years.

5.1 Mitochondrial diversity and phylogeography of African green monkeys

As a first step I used the complete cyt *b* gene to reconstruct the phylogeny of African green monkeys representing a comprehensive data set of faecal, hair and museum samples from sites, which cover most of the genus' wide range (Chapter 2). Based on this data set I gained an overview of the mtDNA diversity within African green monkeys. As inferred from phylogenetic reconstructions the genus can be divided into nine mtDNA clades, representing rather geographic clades than currently recognized taxa, causing several paraphyletic and polyphyletic relationships. These discordances between the geographic distributions of mtDNA clades and morphotypes are most likely outcomes of on-going and past hybridization events, which I will discuss in more detail in section 5.2. Despite the good geographic and taxonomic sample coverage, I was not able to obtain a completely resolved phylogeny using the cyt *b* gene (Chapter 2). However, the cyt *b* gene has been successfully used to reconstruct inter-specific phylogenies of diverse primates and other mammals (Castresana, 2001; Agnarsson & May-Collado, 2008; Roos et al., 2008; Thinh et al., 2010; Tobe et al., 2010). Weakly supported phylogenies might be an indication for the divergence of lineages within a short time period in response to climatic and environmental changes, which has been also found in baboons (Zinner et al., 2009b). Assuming a similar rapid divergence in African green monkeys, it might be that the cyt *b* gene alone does not contain sufficient genetic information to convincingly resolve the phylogeny of the rapidly evolved lineages.

Nonetheless, based on the results of this comprehensive data set, I was able to selectively choose samples to analyse additional mitochondrial loci representing all previously found mtDNA clades. Incorporating this genetic information resulted in better

supported and more consistent branching patterns among statistical approaches and allowed to estimate divergence times (Chapter 4). Although reconstructions of past geographic distributions and dispersal routes often remain hypothetical, since current distributions of mtDNA clades do not necessarily reflect distributions of ancestors of respective clades (Ronquist, 1997), by combining all findings of my thesis a hypothetical phylogeographic scenario can be drawn (Figure 5.1d). Previously, origins of African green monkeys were assumed either in East, South or West Africa (Fig. 5.1a-c; Schwarz, 1926; Hill, 1966; Kingdon, 1984; Elton, 2007; Wertheim & Worobey, 2007). My analyses support a West African origin around 2.46 mya with a subsequent migration to South Africa. This is in general accordance with previous mitochondrial DNA and fossil data (Fig. 5.1c-d; Elton, 2007; Wertheim & Worobey, 2007). However, I was able to retrieve a more detailed picture on potential dispersal patterns, which were not recognized in the previous study (Fig. 5.1c; Wertheim & Worobey, 2007). My data suggest a two-step colonisation of Northeast Africa; first from the West and an independent second from the South (Fig. 5.1d). Based on assumed forest refugia, there are two possible dispersal routes into Southern Africa during dry climatic periods, namely west or east of the eastern Central African forest refuge (Hamilton & Taylor, 1991). Assuming a South African origin, Kingdon (1984) proposed the colonization of the North through the western route (Fig. 5.1b). Although I cannot reject one of these possible dispersal routes into the South, the position of the western *C. tantalus* clade in the mtDNA phylogeny, which currently ranges from East Ghana and Burkina Faso in the West to at least Togo in the East, supports a colonization of Southern Africa via the western route.

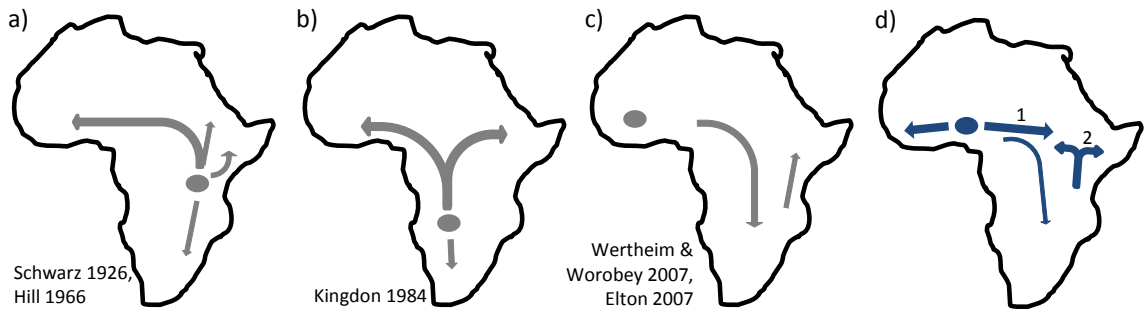

Fig. 5.1 Different phylogeographic scenarios of the African green monkey evolution (a-c) compared to results of the present study (d). Ovals represent presumed origins of the genus. Two independent colonization events of Northeast Africa are indicated by arabic numbers.

The phylogeographic pattern of African green monkeys shows that their current range cannot be simply explained by a "stepping-stone model" (Kimura & Weiss, 1964) from the West to the East and to the South, and the analysis of only few samples of the complete range is not appropriate to disentangle their evolutionary history. This is especially important for the study of co-evolutionary processes of African green monkey species and its specific viruses (Switzer et al., 2005; Wertheim & Worobey, 2007). To obtain reliable results, future studies should include samples of both host and virus representing all mtDNA clades that have been detected in the present study.

5.2 Hybridization and evolution – insights from African green monkeys

One major aim of my thesis was to analyse the degree and pattern of hybridization within African green monkeys. The formation of secondary contact zones and subsequent hybridization between previously isolated populations has been largely attributed to climatic changes during the Pleistocene and Holocene in several organisms (Hewitt, 2011) and results of my thesis suggests a similar picture for African green monkeys.

Based on the analysis of mtDNA several instances of local discordance between distributions of mtDNA clades and morphological traits became obvious, especially in East Africa, where contact zones of four of the currently six recognized species exist (Chapter 2). Most of these patterns support cases of observed on-going hybridization (Napier, 1981; Kingdon, 1997; Mekonnen et al., 2012). Beside this, I also found polyphylies derived from samples beyond contemporary hybrid zones, which might be indicative of ancient introgressive hybridization. Although, past introgressive hybridization is also known from several other primate species and seems to be common in mammals in general (Arnold, 1997; Zinner et al., 2011a), discordant patterns can also derive from other evolutionary processes like incomplete lineage sorting, homoplasy or hemiplasy. Therefore, the recognition of mitochondrial introgression requires the additional analysis of nuclear DNA (e.g., Funk & Omland, 2003; Avise & Robinson, 2008). Therefore I analysed two Y-chromosomal markers to evaluate, if polyphylies in the phylogeny of African green monkeys represent outcomes of ancient introgressive hybridization events (Chapter 3). Based on these data, I found that not all

incongruences beyond current contact zones can be explained by past introgression. Surprisingly, I found two distinct Y-chromosomal haplotypes within the distribution of *Chlorocebus tantalus* which are in accordance with mtDNA data, thus rejecting the hypothesis of ancient hybridization for this particular case. In contrast, my data suggest that the currently recognized species *C. tantalus* represents two separate taxa (possibly species) due to cryptic genetic variation (Chapter 3).

A different scenario became obvious for *C. pygerythrus*. This species ranges from the Republic of South Africa to southern Ethiopia and Somalia. Analyses of mtDNA indicated that morphotypes of *C. pygerythrus* from different geographic regions are scattered in three distinct clades causing polyphyletic relationships (Chapter 3). In contrast to mtDNA, Y-chromosomal analyses of samples from Southeast Ethiopia and Kenya/Tanzania revealed identical haplotypes, assuming that polyphyletic relationships within *C. pygerythrus* are the result of ancient introgressive hybridization. Due to male biased dispersal in African green monkeys mitochondrial capture is less likely and nuclear swamping by male introgression is the most reasonable explanation to have caused this pattern (Zinner et al., 2011a). Although introgressive hybridization usually does not produce a change in species diversity in contrast to other hybridization processes (e.g., hybrid speciation or amalgamation), persistent nuclear swamping by male introgression can lead to the complete cytonuclear extinction of the resident species (Zinner et al., 2009b, 2011a). Due to the distinctiveness of the mtDNA clade from Southeast Ethiopia and its basal position in the phylogeny of *Chlorocebus*, I assume that this *C. pygerythrus* population carries relict mitochondrial genomes of a cytonuclear extinct taxon or population. Unfortunately, I was not able to obtain a high quality sample of a male individual from Southern Africa impeding the analysis of Y-chromosomal data from the southernmost *C. pygerythrus* clade. However, since the *C. pygerythrus* mtDNA from Kenya and Tanzania falls basal to *C. aethiops* and *C. tantalus* in the phylogeny, I assume that this clade is also the result of male biased ancient introgression from *C. pygerythrus* into an ancestral form of *C. aethiops* and *C. tantalus*. However, the longer the time since the last gene exchange, the more difficult is the determination of the native mtDNA clade of a species (Funk & Omland, 2003). To fully resolve directions of introgression from one taxon into the other and to determine,

which mtDNA clade represents the "true" mitochondrial genome of *C. pygerythrus*, further nuclear DNA analyses of more samples including the southernmost mtDNA clade are necessary.

MtDNA and Y-chromosomal data provide evidence of ancient and on-going hybridization in contact zones of all African green monkey species. An exception is the West African contact zone of *C. sabaeus* and *C. tantalus* which extends along the Volta River from South Ghana to Burkina Faso and probably even farther North into southeastern Mali (Lernould, 1988). The lack of hybridization among these species suggests that these populations have been separated for a longer period and that the Volta River and its confluents even today represent geographic barriers. Surprisingly, evidence for hybridization was detected in a single population in South Ghana West of the Volta River. Here, an exceptional *C. tantalus* population that is found west of the Volta River carries the mitochondrial genome of *C. sabaeus*. Since the Volta River in this region is a wide river which might constitute an even stronger natural barrier, a possible explanation for the finding might be that the exchange of populations across the Volta River in South Ghana was induced by humans. Anthropological impact is highly probable because African green monkeys are often kept as pets and humans have settled in the area along the Atlantic coast and likely crossed the river frequently.

What do these results tell us about hybridization in primates (and animals) in general? On-going as well as ancient introgression seems to be widespread in African green monkeys. Therefore, African green monkeys represent one more vertebrate and primate genus, whose evolutionary history has been and is still impacted by hybridization. Although it is no longer questioned that hybridization is common in animals including primates and even us humans, there are still debates on how introgressive hybridization acts on genetic and species diversity as well as on speciation and evolution (Arnold, 1997; Abbott et al., 2013). Major questions are if introgression enhances genetic diversity and thereby adaptive evolution, or if it reflects the transmission of only neutral characters, due to the movement of the hybrid zone and unidirectional sex-specific hybridization, and if it rather leads to the formation of less fit individuals compared to parental forms (Arnold, 1997; Seehausen, 2013). My results indicate that in some cases local populations were completely wiped out due to nuclear

swamping by populations of another species. On the one hand, this has led to the cytonuclear extinction of a former taxon, but at the same time it increased the genetic variability within the invasive species, which carries now foreign mitochondrial genomes in certain regions of its geographic distribution. Although the analysis of non-recombining markers is appropriate to elucidate sex-specific introgression patterns, it does not allow any conclusions on the admixture of the nuclear genome. Therefore, the possibility remains that besides the transmission of the mitochondrial genome also other genomic elements became admixed in involved African green monkey taxa leading to a mosaic genome (Arnold, 1997; Abbott et al., 2013). The evolutionary capability of genome admixture is probably best shown by the introgression of archaic alleles from Denisovans, which are now involved in the immune system of modern Eurasian humans (Abi-Rached et al., 2011).

From the conservation perspective it is argued, that introgression between species in secondary contact zones is the result of a loss in environmental heterogeneity and leads to extinction, which is nowadays often caused by anthropogenic influences (Wolf et al., 2001; Seehausen et al., 2008). Similar concerns exist over the endemic species *C. djamdjamensis*, which is restricted to the Southern highlands of Ethiopia and exhibits some specific ecological characters compared to other African green monkey species (Kingdon, 1997; Mekonnen et al., 2010a). On-going hybridization with neighbouring lowland species *C. aethiops* and *C. pygerythrus* due to progressive deforestation is assumed to represent a threat to the survival of this "vulnerable" species (Kingdon, 1997; Butynski et al. 2008; Mekonnen et al., 2012). My molecular data support the hypothesis of contemporary hybridization among *C. aethiops* and *C. djamdjamensis*. Based on mtDNA of the holotype of *C. djamdjamensis* hybridization among these two species occurs at least since more than 100 years ago (Chapter 2). Although my data revealed only *C. djamdjamensis*-like morphotypes carrying mtDNA of *C. aethiops*, the cytonuclear extinction of *C. djamdjamensis* by persisting introgression and on-going habitat disturbance might be a possible scenario due to the comparatively low population size of *C. djamdjamensis* (Cordingley et al., 2009; Mekonnen et al., 2010b). Although, introgressive hybridization is often favoured by anthropogenic impact (Detwiler et al., 2005), results of the present thesis indicate that ancient introgressive

hybridization has occurred naturally throughout the evolution of African green monkeys, which is likely a result of recurrent range retraction and extension in response to Pleistocene climatic changes.

5.3 Major trends in the evolution of African savannah mammals

The second major aim of my thesis was to use African green monkeys as a model to examine general evolutionary trends in African savannah mammals. Based on divergence time estimates, the evolution of African green monkeys began around 2.46 mya (Chapter 4), and hence, the analysis of their phylogeography offers a promising possibility to evaluate evolutionary influences of major climatic and environmental changes in African savannahs back to the Early Pleistocene. To get a broader picture I compared the phylogeography of African green monkeys to phylogeographies of three other widely distributed savannah mammals by applying similar methods to previously published sequence data of baboons (*Papio*), hartebeests (*Alcelaphus*) and warthogs (*Phacochoerus*) (Chapter 4).

Based on findings of this comparative phylogeographic approach, major divergences of savannah mammals occurred not only during humid periods, but also during more arid periods, when savannah areas were likely diminished by the advance of desert regions (Nichol, 1999; Scholz et al., 2007). Although comparisons of divergence times and paleoclimatic data must be treated carefully (see below), this result challenges the existence of savannah refugia during only warm-humid periods, when equatorial rain forests expanded their ranges (Flagstadt, 2001; Hassanin et al., 2007; Lorenzen et al., 2012). However, current distribution limits of major biogeographic clades in West, East and South Africa indicate that respective populations became most probably separated in regions in north central Africa (e.g., Chad) and southeast Africa (e.g., South Tanzania), which are known to have been influenced by extreme humid as well as extreme dry climatic conditions within the Pleistocene and Holocene (Chapter 4; Nichol, 1999; Goudie, 2005; Scholz et al., 2007; Cowling et al., 2008; Dupont et al., 2011; Zinner et al., 2011b). Therefore, it is likely that savannah refugia existed during both periods of extreme wetness and drought, which led to recurrent separations of

savannah mammal populations in respective refugia and to the possible interference of these events in the evolutionary history of savannah mammals.

Furthermore, in accordance with a recent hypothesis, I intended to test if more regional African climate variation superimposed on the global climatic trend was a major driver in the evolution of African savannah mammals. Based on paleontological data significant humid periods occurred in East Africa during the otherwise increasing global arid trend assuming that the African climate variation was less influenced by the Northern Hemisphere glacial cycles (Larrasoana et al., 2003; Lourens et al., 2004; Trauth et al., 2005, 2007, 2009). These findings led to a new hypothesis on the evolution of early hominins in East Africa, and it was assumed that more regional African climates played also major roles in the evolution of other savannah mammals (Trauth et al., 2010). A comparative analysis of population histories of the four considered mammal genera indicated that changes of population sizes during the last 500,000 years did not coincide with climate extremes of the Northern Hemisphere glacial cycles, neither with the Last Interglacial nor with the Last Glacial Maximum. Therefore, my data suggest that a more regional climate variation affected the evolution of African savannah mammals (Chapter 4).

The analysis of time estimates within mitochondrial phylogenies or population histories based on fossil calibration can be erroneous due to differences in instantaneous mutation rates and long-term substitution rates, as well as due to effects of mitochondrial saturation (Ho et al., 2005, 2011; Lukoschek et al., 2012). Moreover, divergence time estimates within ungulate genera resulted in time estimates with broad confidence intervals, which can be explained by the application of high standard deviations to calibrated nodes (Chapter 4). Based on these uncertainties in time estimates any comparison of temporal patterns must be treated with caution. However, with respect to the phylogenetic patterns within primate genera I do not expect a more conclusive picture among divergences and humid periods irrespective of substitution rates or calibration nodes that are applied, while in ungulate genera a more concordant picture among major divergences and humid periods might be conceivable. In the optimal case, the analysis of additional nuclear markers in a separate data set and the

critical evaluation of several candidate fossil calibrations are necessary to obtain more reliable and comparable results (Lukoschek et al., 2012).

The intention of this part of my study was to use African green monkeys as a model to evaluate general evolutionary trends in the evolution of African savannah mammals. Irrespective of substitution rates, patterns of past population size changes showed dissimilarities between primates and ungulates. Whereas data on both primate genera indicate population declines within last 500,000 years, except for the southern baboon clade, ungulate genera showed an overall increase in population sizes in the same period (Chapter 4). Although compared taxa exhibit currently remarkable similar geographic distributions in African savannahs, responses to climatic changes depend largely on species specific adaptations and tolerance to environmental changes (Stewart et al., 2010). Both ungulate genera are predominantly grazers and well adapted to open grasslands. However, even though African green monkeys and baboons are well adapted to the open savannah habitat compared to forest living primates, they still depend on the occurrence of fruiting trees as main food resource (Kingdon, 1997). Ecological differences among taxa as in this case between grazers and frugivores represent a drawback in comparative phylogeographic approaches, but at the same time, they accentuate the importance of the analysis of mammalian taxa of different guilds to obtain a more conclusive picture of the impact of climatic and environmental changes on the savannah biome.

5.4 African green monkeys – species or subspecies?

Although the intention of my thesis was not to infer taxonomic conclusions, results of this thesis inevitably pose the question how African green monkeys should be classified taxonomically. This question is especially motivating since actually, a debate on different species concepts has arisen again, criticising recent trends towards taxonomic "oversplitting" and "species inflation" (Markolf et al., 2011; Zachos et al., 2012; Gippoliti & Groves, 2013; Groves, 2013). In accordance with the primate taxonomy of Groves (2001; 2005), who largely adopts the Phylogenetic Species Concept (PSC), I considered six African green monkey species based on morphological differences (see Chapter 1 and 2 for more details). However, other researchers treat these species as subspecies of

C. aethiops (Grubb et al., 2003; Elton et al., 2010). Results of my thesis indicate even nine similarly distinct mtDNA clades that do not correspond to any recent taxonomy (Chapter 2). However, since I found evidence for on-going as well as ancient hybridization among species, the diversity of maternally inherited mtDNA alone cannot be used for taxonomic inferences. Unfortunately, my data set did not include samples of male individuals of appropriate quality from all recognized taxa and mtDNA clades, preventing me from the reconstruction of a complete Y-chromosomal phylogeny of African green monkeys. Nonetheless, based on the analysis of available samples I found that the species diversity is likely better reflected by Y-chromosomal haplotypes than by mtDNA variation. Revisiting these results I argue that based on morphological characters as well as on the genetic diversity there is enough evidence for the recognition of the six currently acknowledged species of African green monkeys. Since species are the units of conservation, this classification is particularly relevant for the endemic species *C. djamdjamensis*, which is the only species of this genus that is listed as "vulnerable" (Butynski et al., 2008).

The most unexpected result of my thesis was the detection of a possible new morphologically cryptic taxon within the range of the currently recognized species *C. tantalus*. However, due to missing detailed morphological data I do not propose any taxonomical changes yet. Moreover, prior to any taxonomic consideration I recommend analyses of further nuclear markers including samples from holotypes of *tantalus* and *chrysurus* (currently a synonym of *C. sabaeus* (Groves, 2001) or *C. tantalus* (Hill, 1966); see Chapter 3 for a more detailed discussion). Only with these additional data appropriate taxonomic conclusions can be drawn. If these data support my present findings, this taxon might represent a new African green monkey species.

5.5 Conclusions

African green monkeys represent morphologically and genetically a diverse primate genus that evolved in African savannahs with the beginning of the Pleistocene. Its complex phylogeography with the formation of secondary contact zones and subsequent hybridization provides evidence for recurrent range retraction and expansion of populations in response to climatic and environmental changes within the

last 2.5 million years. Widespread on-going and ancient introgressive hybridization in African green monkeys highlights the important influence of hybridization on the evolution of primates and animals in general. Comparative analyses with three other widely distributed genera indicate that population ranges of African savannah mammals were similarly affected by extreme warm-humid and cold-arid climate periods. Furthermore, results of the comparative approach support the hypothesis that the evolution of African savannah mammals has been rather influenced by more regional African climate variations than by Northern Hemisphere glacial cycles. While my thesis demonstrates the importance of the analysis of mtDNA on the one hand, it also indicates the need of Y-chromosomal analyses in addition to mtDNA to obtain a more conclusive picture of phylogeographic patterns, hybridization, and of genetic and species diversity.

5.6 Outlook

Although the results of my thesis provide a good overview of the mitochondrial diversity and phylogeography of African green monkeys, there are still regions, especially in Southern Africa, which needs to be sampled and incorporated in future studies. To get a more detailed picture of the evolution of African savannah mammals in general, more molecular data are needed of various mammalian taxa, to diminish uncertainties in divergence time estimations and to be able to consider differences in responses to environmental changes due to different ecological adaptations.

Beside this, the Y-chromosomal data set included samples of only few African green monkeys species and did not allow the reconstruction of the phylogeny of the complete genus, which would certainly improve the understanding of hybridization within African green monkeys. In my thesis I analysed maternal and paternal inherited markers, which were advantageous over recombining loci to examine sex-specific introgression patterns. However, as I mentioned above, the analysis of diverse nuclear loci (e.g., genome wide Single Nucleotide Polymorphisms [SNPs]) might provide additional information on patterns of hybridization and potential admixture of certain gene material. Thereby, future studies should focus on the possible new taxon (or species) within the range of *C. tantalus* to test if this finding can be verified by the

analysis of additional nuclear DNA and morphological characters and to determine its geographical range.

REFERENCES

Abbott R., Albach D., Ansell S., Arntzen J.W., Baird S.J.E., Bierne N., Boughman J., Brelsford a., Buerkle C. a., Buggs R., Butlin R.K., Dieckmann U., Eroukhmanoff F., Grill a., Cahan S.H., Hermansen J.S., Hewitt G., Hudson a. G., Jiggins C., Jones J., Keller B., Marczewski T., Mallet J., Martinez-Rodriguez P., Möst M., Mullen S., Nichols R., Nolte a. W., Parisod C., Pfennig K., Rice a. M., Ritchie M.G., Seifert B., Smadja C.M., Stelkens R., Szymura J.M., Väinölä R., Wolf J.B.W., & Zinner D. (2013) Hybridization and speciation. *Journal of Evolutionary Biology*, **26**, 229–246.

Abi-Rached L., Jobin M.J., Kulkarni S., McWhinnie A., Dalva K., Gragert L., Babrzadeh F., Gharizadeh B., Luo M., Plummer F. a, Kimani J., Carrington M., Middleton D., Rajalingam R., Beksac M., Marsh S.G.E., Maiers M., Guethlein L. a, Tavoularis S., Little A.-M., Green R.E., Norman P.J., & Parham P. (2011) The shaping of modern human immune systems by multiregional admixture with archaic humans. *Science*, **334**, 89–94.

Agnarsson I. & May-Collado L.J. (2008) The phylogeny of Cetartiodactyla: the importance of dense taxon sampling, missing data, and the remarkable promise of cytochrome b to provide reliable species-level phylogenies. *Molecular Phylogenetics and Evolution*, **48**, 964–985.

Allal F., Sanou H., Millet L., Vaillant A., Camus-Kulandaivelu L., Logossa Z., Lefèvre F., & Bouvet J.-M. (2011) Past climate changes explain the phylogeography of Vitellaria paradoxa over Africa. *Heredity*, **107**, 174–186.

Alpers D.L., Van Vuuren B.J., Arctander P., & Robinson T.J. (2004) Population genetics of the roan antelope (*Hippotragus equinus*) with suggestions for conservation. *Molecular Ecology*, **13**, 1771–1784.

Anandam M.V., Bennett E.L., Davenport T.R.B., Davies N.J., Detwiler K.M., Engelhardt A., Eudey A.A., Gadsby E.L., Groves C.P., Healy A., Karanth K.P., Molur S., Nadler T., Richardson M.C., Riley E.P., Roos C., Rylands A.B., Sheeran L.K., Ting N., Wallis J., Waters S.S., Whittaker D.J., Zinner D. (in press). *Species accounts of Cercopithecidae.* In: Mittermeier RA, Wilson DE (eds.) Handbook of the Mammals of the World. Vol. 3 Primates. Lynx Edicions, Barcelona.

Andersen K.K., Svensson A., Johnsen S.J., Rasmussen S.O., Bigler M., Röthlisberger R., Ruth U., Siggaard-Andersen M.-L., Peder Steffensen J., & Dahl-Jensen D. (2006) The Greenland Ice Core Chronology 2005, 15–42ka. Part 1: constructing the time scale. *Quaternary Science Reviews*, **25**, 3246–3257.

Anthony N.M., Johnson-Bawe M., Jeffery K., Clifford S.L., Abernethy K. a, Tutin C.E., Lahm S. a, White L.J.T., Utley J.F., Wickings E.J., & Bruford M.W. (2007) The role of Pleistocene refugia and rivers in shaping gorilla genetic diversity in central Africa. *Proceedings of the National Academy of Sciences*, **104**, 20432–20436.

Arctander P., Johansen C., & Coutellec-Vreto M.A. (1999) Phylogeography of three closely related African bovids (tribe Alcelaphini). *Molecular Biology and Evolution*, **16**, 1724–1739.

Arnold M.L. (1997) *Natural Hybridization and Evolution.* Oxford University Press, New York.

Arnold M.L. (2006) *Evolution Through Genetic Exchange.* Oxford University Press, New York.

Arnold M.L. & Meyer A. (2006) Natural hybridization in primates: one evolutionary mechanism. *Zoology*, **109**, 261–276.

Avise J.C. (1998) The history and purview of phylogeography: a personal reflection. *Molecular Ecology*, **7**, 371–379.

Avise J.C. (2004) *Molecular Markers, Natural History, and Evolution.* Sinauer Associates, Sunderland, MA.

Avise J.C. (2009) Phylogeography: retrospect and prospect. *Journal of Biogeography*, **36**, 3–15.

Avise J.C. & Robinson T.J. (2008) Hemiplasy: a new term in the lexicon of phylogenetics. *Systematic Biology*, **57**, 503–507.

Baker A.J. (2008) Islands in the sky: the impact of Pleistocene climate cycles on biodiversity. *Journal of Biology*, **7**, 32.

Bandelt H., Forster P., & Rohl A. (1999) Median-joining networks for inferring intraspecific phylogenies. *Molecular Biology and Evolution*, **16**, 37–48.

Basell L. (2008) Middle Stone Age (MSA) site distributions in eastern Africa and their relationship to Quaternary environmental change, refugia and the evolution of *Homo sapiens*. *Quaternary Science Reviews*, **27**, 2484–2498.

Belsky A.J. (1990) Tree/grass ratios in East African savannas: a comparison of existing models. *Journal of Biogeography*, **17**, 483–489.

Bermingham E. & Moritz C. (1998) Comparative phylogeography: concepts and applications. *Molecular Ecology*, **7**, 367–369.

Bernatchez L. & Wilson C. (1998) Comparative phylogeography of Nearctic and Palearctic fishes. *Molecular Ecology*, **7**, 431–452.

Bertola L.D., Van Hooft W.F., Vrieling K., Uit de Weerd D.R., York D.S., Bauer H., Prins H.H.T., Funston P.J., Udo de Haes H.A., Leirs H., Van Haeringen W.A., Sogbohossou E., Tumenta P.N., & De Iongh H.H. (2011) Genetic diversity, evolutionary history and

implications for conservation of the lion (*Panthera leo*) in West and Central Africa. *Journal of Biogeography*, **38**, 1356–1367.

Blyth E. (1845) Notices of various Mammalia, with Descriptions of many new Species. *The Annals and Magazine of Natural History*, **15**, 461.

Booth A. (1956) The distribution of primates in the Gold Coast. *Journal of the West African Science Association*, **2**, 122–133.

Booth A. (1958) The zoogeography of West African primates: a review. *Bull I'lFAN*, **20**, 587–622.

Brochmann C., Gabrielsen T.M., Nordal I., Landvik J.Y., & Elven R. (2003) Glacial survival or *tabula rasa*? The history of North Atlantic biota revisited. *Taxon*, **52**, 417–450.

Brown D.M., Brenneman R. a, Koepfli K.-P., Pollinger J.P., Milá B., Georgiadis N.J., Louis E.E., Grether G.F., Jacobs D.K., & Wayne R.K. (2007) Extensive population genetic structure in the giraffe. *BMC Biology*, **5**, e57.

Brown W.M., Prager E.M., Wang A., & Wilson A.C. (1982) Mitochondrial DNA sequences of primates tempo and mode of evolution. *Journal of Molecular Evolution*, **18**, 225–239.

Brunet M., Guy F., Pilbeam D., Lieberman D., Likius A., Mackaye H., Ponce deLeón M., Zollokofer C., & Vignaud P. (2005) New material of the earliest hominid from the Upper Miocene of Chad. *Nature*, **434**, 752–755.

Buggs R.J.A. (2007) Empirical study of hybrid zone movement. *Heredity*, **99**, 301–12.

Bush M.B. & De Oliveira P.E. (2006) The rise and fall of the Refugial Hypothesis of Amazonian speciation: a paleo-ecological perspective. *Biota Neotropica*, **6**, .

Butynski T.M. (2008) *Chlorocebus cynosuros*. *IUCN Red List of Threatened Species.* Version 2012.1. Available at <www.iucnredlist.org>. Downloaded on 03 August 2012.

Butynski T.M., Gippoliti S., Kingdon J., de Jong Y. (2008) *Chlorocebus djamdjamensis*. *IUCN Red List of Threatened Species.* Version 2012.1. Available at <www.iucnredlist.org>. Downloaded on 03 August 2012.

Carstens B.C. & Richards C.L. (2007) Integrating coalescent and ecological niche modeling in comparative phylogeography. *Evolution*, **61**, 1439–1454.

Castresana J. (2000) Selection of conserved blocks from multiple alignments for their use in phylogenetic analysis. *Molecular Biology and Evolution*, **17**, 540–552.

Castresana J. (2001) Cytochrome b phylogeny and the taxonomy of great apes and mammals. *Molecular Biology and Evolution*, **18**, 465–471.

Cerling T.E. (1992) Development of grasslands and savannas in East Africa during the Neogene. *Palaeography, Palaeoclimatology, Palaeoecology*, **97**, 241–247.

Cerling T.E., Wynn J.G., Andanje S.A., Bird M.I., Korir D.K., Levin N.E., Mace W., Macharia A.N., Quade J., & Remien C.H. (2011) Woody cover and hominin environments in the past 6 million years. *Nature*, **476**, 51–56.

Charpentier M.J.E., Fontaine M.C., Cherel E., Renoult J.P., Jenkins T., Benoit L., Barthès N., Alberts S.C., & Tung J. (2012) Genetic structure in a dynamic baboon hybrid zone corroborates behavioural observations in a hybrid population. *Molecular Ecology*, **21**, 715–31.

Cordingley J.E., Sundaresan S.R., Fischhoff I.R., Shapiro B., Ruskey J., & Rubenstein D.I. (2009) Is the endangered Grevy's zebra threatened by hybridization? *Animal Conservation*, **12**, 505–513.

Cowling S.A., Cox P.M., Jones C.D., Maslin M.A., Peros M., & Spall S.A. (2008) Simulated glacial and interglacial vegetation across Africa: implications for species phylogenies and trans-African migration of plants and animals. *Global Change Biology*, **14**, 827–840.

Dandelot P. (1959) Note sur la classification des cercopithèques du groupe *aethiops*. *Mammalia*, **23**, 357–368.

Dandelot P. (1971) Order Primates. *The mammals of Africa: an identification manual. Part 3* (ed. by J. Meester and H. Setzer), pp. 1–29. Smithsonian Institution Press, Washington DC.

Dandelot P. & Prévost J. (1972) Contribution à l'étude des primates d'Éthiopie (simiens). *Mammalia*, **36**, 607–633.

Darwin C. (1859) *On the Origin of Species by Means of Natural Selection or the Preservation of Favoured Races in the Struggle for Life.* John Murray, London.

de Jong Y.A. & Butynski T.M. (2010) Three Sykes's monkey *Cercopithecus mitis* × vervet monkey *Chlorocebus pygerythrus* hybrids in Kenya. *Primate Conservation*, **25**, 43–56.

Delson E. (1980) Fossil macaques, phyletic relationships and a scenario of deployment. *The macaques: Studies in Ecology, Behaviour and Evolution* (ed. by D. Lindburg), pp. 10–33. Van Nostrand Reinhold Company, New York.

Delson E. (2000) Cercopithecinae. *Encyclopedia of Human Evolution and Prehistory* (ed. by E. Delson, I. Tattersall, J. Van Couvering, and A. Brooks), pp. 166–171. Garland Publishing Inc., New York.

deMencoal P.B. (1995) Plio-Pleistocene African climate. *Science*, **270**, 53–59.

deMenocal P.B. (2004) African climate change and faunal evolution during the Pliocene–Pleistocene. *Earth and Planetary Science Letters*, **220**, 3–24.

Detwiler K.M., Burrell A.S., & Jolly C.J. (2005) Conservation implications of hybridization in African Cercopithecine monkeys. *International Journal of Primatology*, **26**, 661–684.

Dowling T. & DeMarais B. (1993) Evolutionary significance of introgressive hybridization in cyprinid fishes. *Nature*, **362**, 444-446.

Drummond A., Ashton B., Buxton S., Cheung M., Cooper A., Duran C., Field M., Heled J., Kearse M., Markowith S., Moir R., Stones-Havas S., Sturrock S., Thierer T., & Wilson A. (2011) *Geneious v5.4.* Available at: www.geneious.com.

Drummond A., Rambaut A., Shapiro B., & Pybus O.G. (2005) Bayesian coalescent inference of past population dynamics from molecular sequences. *Molecular Biology and Evolution*, **22**, 1185–1192.

Drummond A.J., Ho S.Y.W., Phillips M.J., & Rambaut A. (2006) Relaxed phylogenetics and dating with confidence. *PLoS Biology*, **4**, e88.

Drummond A.J. & Rambaut A. (2007) BEAST: Bayesian evolutionary analysis by sampling trees. *BMC Evolutionary Biology*, **7**, e214.

Dupont L.M., Caley T., Kim J.-H., Castañeda I., Malaizé B., & Giraudeau J. (2011) Glacial-interglacial vegetation dynamics in South Eastern Africa coupled to sea surface temperature variations in the Western Indian Ocean. *Climate of the Past*, **7**, 1209–1224.

Ehrich D., Gaudeul M., Assefa A., Koch M. a, Mummenhoff K., Nemomissa S., & Brochmann C. (2007) Genetic consequences of Pleistocene range shifts: contrast between the Arctic, the Alps and the East African mountains. *Molecular Ecology*, **16**, 2542–2559.

Elton S. (2007) Environmental correlates of the cercopithecoid radiations. *Folia Primatologica*, **78**, 344–364.

Elton S., Dunn J., & Cardini A. (2010) Size variation facilitates population divergence but does not explain it all : an example study from a widespread African monkey. *Biological Journal of the Linnean Society*, **101**, 823–843.

Engelberger S. (2010) *Annotated catalogue of primate type specimens in the mammal collection of the Museum of Natural History Vienna,*M.Sc. thesis, Universität Wien, Vienna.

Fernández M.H. & Vrba E.S. (2005) A complete estimate of the phylogenetic relationships in Ruminantia: a dated species-level supertree of the extant ruminants. *Biological Reviews*, **80**, 269–302.

Fjeldsa J.O.N. & Lovett J.O.N.C. (1997) Geographical patterns of old and young species in African forest biota: the significance of specific montane areas as evolutionary centres. *Biodiversity and Conservation*, **6**, 325–346.

Flagstad O., Syvertsen P.O., Stenseth N.C., & Jakobsen K.S. (2001) Environmental change and rates of evolution: the phylogeographic pattern within the hartebeest complex as related to climatic variation. *Proceedings of the Royal Society London B*, **268**, 667–677.

Fontanesi L., Tazzoli M., & Russo V. (2007) Non-invasive and simple methods for sampling rabbit DNA for PCR analysis of melanocortin 1 receptor (MC1R) gene mutations: a technical note. *World Rabbit Science*, **15**, 121–126.

Fouquet A., Noonan B.P., Rodrigues M.T., Pech N., Gilles A., & Gemmell N.J. (2012) Multiple quaternary refugia in the eastern Guiana shield revealed by comparative phylogeography of 12 frog species. *Systematic Biology*, **61**, 461–489.

Funk D.J. & Omland K.E. (2003) Species-level paraphyly and polyphyly: frequency, causes, and consequences, with insights from animal mitochondrial DNA. *Annual Review of Ecology, Evolution, and Systematics*, **34**, 397–423.

Gippoliti S. & Groves C.P. (2013) "Taxonomic inflation" in the historical context of mammalogy and conservation. *Hystrix*, doi:10.4404/hystrix-23.2-8685.

Gongora J., Cuddahee R.E., Nascimento F.F. Do, Palgrave C.J., Lowden S., Ho S.Y.W., Simond D., Damayanti C.S., White D.J., Tay W.T., Randi E., Klingel H., Rodrigues-Zarate C.J., Allen K., Moran C., & Larson G. (2011) Rethinking the evolution of extant sub-Saharan African suids (Suidae, Artiodactyla). *Zoologica Scripta*, **40**, 327–335.

Goudie A.S. (2005) The drainage of Africa since the Cretaceous. *Geomorphology*, **67**, 437–456.

Green R.E., Krause J., Briggs A.W., Maricic T., Stenzel U., et al. (2010) A draft sequence of the Neandertal genome. *Science*, **328**, 710–722.

Groves C. (2001) *Primate Taxonomy.* Smithonian Institution Press, Washington DC.

Groves C. (2005) Order primates. *Mammal Species of the World: a Taxonomic and Geographic Reference* (ed. by D. Wilson and D. Reeder), pp. 111–184. Johns Hopkins University Press, Baltimore.

Groves C.P. (2013) The nature of species: A rejoinder to Zachos et al. *Mammalian Biology - Zeitschrift für Säugetierkunde*, **78**, 7–9.

Grubb P. (1982) Refuges and dispersal in the speciation of African forest mammals. *Biological Diversification in the Tropics* (ed. by G.T. Prance), pp. 537–553. Columbia University Press, New York.

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.

Guindon S. & Gascuel O. (2003) A simple, fast, and accurate algorithm to estimate large phylogenies by maximum likelihood. *Systematic Biology*, **52**, 696–704.

Haffer J. (1969) Speciation in amazonian forest birds. *Science*, **165**, 131–137.

Haldane J.B.S. (1922) Sex ratio and unisexual sterility in hybrid animlas. *Journal of Genetics*, **12**, 101–109.

Hamilton A. & Taylor D. (1991) History of climate and forests in tropical Africa during the last 8 million years. *Climatic Change*, **19**, 65–78.

Hassanin A., Delsuc F., Ropiquet A., Hammer C., Jansen van Vuuren B., Matthee C., Ruiz-Garcia M., Catzeflis F., Areskoug V., Nguyen T.T., & Couloux A. (2012) Pattern and timing of diversification of Cetartiodactyla (Mammalia, Laurasiatheria), as revealed by a comprehensive analysis of mitochondrial genomes. *Comptes Rendus Biologies*, **335**, 32–50.

Hassanin A. & Douzery E.J. (1999) The tribal radiation of the family Bovidae (Artiodactyla) and the evolution of the mitochondrial cytochrome b gene. *Molecular Phylogenetics and Evolution*, **13**, 227–243.

Hassanin A. & Ropiquet A. (2004) Molecular phylogeny of the tribe Bovini (Bovidae, Bovinae) and the taxonomic status of the Kouprey, Bos sauveli Urbain 1937. *Molecular Phylogenetics and Evolution*, **33**, 896–907.

Hassanin A., Ropiquet A., Gourmand A.-L., Chardonnet B., & Rigoulet J. (2007) Mitochondrial DNA variability in Giraffa camelopardalis: consequences for taxonomy, phylogeography and conservation of giraffes in West and central Africa. *Comptes Rendus Biologies*, **330**, 265–74.

Haug G.H., Tiedemann R., Zahn R., & Ravelo A.C. (2001) Role of Panama uplift on oceanic freshwater balance. *Geology*, **29**, 207–210.

Haus T., Akom E., Agwanda B., Hofreiter M., Roos C., & Zinner D. (2013) Mitochondrial Diversity and Distribution of African Green Monkeys (*Chlorocebus* Gray, 1870). *American Journal of Primatology*, **75**, 350–360.

Haus, T., Hofreiter, M., Roos, C., Zinner, D. (submitted). Geographical but not temporal concordance in the genetic structure of African savannah mammals.

Heled J. & Drummond A.J. (2008) Bayesian inference of population size history from multiple loci. *BMC Evolutionary Biology*, **8**, e289.

Herman J.S. & Searle J.B. (2011) Post-glacial partitioning of mitochondrial genetic variation in the field vole. *Proceedings of the Royal Society B*, **278**, 3601–3607.

Hewitt G.M. (2004) The structure of biodiversity - insights from molecular phylogeography. *Frontiers in zoology*, **1**, e4.

Hewitt G.M. (2011) Quaternary phylogeography: the roots of hybrid zones. *Genetica*, **139**, 617–638.

Hill W.C.O. (1966) *Primates: comparative anatomy and taxonomy. VI. Catarrhini, Cercopithecoidea, Cercopithecinae.* Edingburgh University Press, Edingburgh.

Ho S.Y.W., Lanfear R., Bromham L., Phillips M.J., Soubrier J., Rodrigo A.G., & Cooper A. (2011) Time-dependent rates of molecular evolution. *Molecular Ecology*, **20**, 3087–3101.

Ho S.Y.W., Phillips M.J., Cooper A., & Drummond A.J. (2005) Time dependency of molecular rate estimates and systematic overestimation of recent divergence times. *Molecular biology and evolution*, **22**, 1561–1568.

Ho S.Y.W., Saarma U., Barnett R., Haile J., & Shapiro B. (2008) The effect of inappropriate calibration: three case studies in molecular ecology. *PloS ONE*, **3**, e1615.

Van Hooft W.F., Groen A.F., & Prins H.H.T. (2002) Phylogeography of the African buffalo based on mitochondrial and Y-chromosomal loci: Pleistocene origin and population expansion of the Cape buffalo subspecies. *Molecular Ecology*, **11**, 267–279.

Huelsenbeck J., Ronquist F., Nielsen R., & Bollback J. (2001) Bayesian inference of phylogeny and its impact on evolutionary biology. *Science*, **294**, 2310–2314.

IUCN. 2012. *IUCN Red List of Threatened Species*. Version 2012.1. <www.iucnredlist.org>. Downloaded on 15 October 2012.

Jaffe K.E. & Isbell L.A. (2011) The guenons: polyspecific associations in socioecological perspective. *Primates in Perspective* (ed. by C.J. Campbell, A. Fuentes, K.C. MacKinnon, S.K. Bearder, and R.M. Stumpf), pp. 277–300. Oxford University Press, New York.

Janis C.M. (1993) Tertiary mammal evolution in the context of changing climates, vegetation, and tectonic events. *Annual Review of Ecology and Systematics*, **24**, 467–500.

Johnsen S.J., Clausen H.B., Dansgaard W., Fuhrer K., Gundestrup N., Hammer C.U., Iversen P., Jouzel J., Stauffer B., & Steffensen J.P. (1992) Irregular glacial interstadials recorded in a new Greenland ice core. *Nature*, **359**, 311–313.

Jolly C.J., Burrell a S., Phillips-Conroy J.E., Bergey C., & Rogers J. (2011) Kinda baboons (*Papio kindae*) and grayfoot chacma baboons (*P. ursinus griseipes*) hybridize in the Kafue river valley, Zambia. *American journal of primatology*, **73**, 291–303.

Juergens N. (1997) Floristic biodiversity and history of African arid regions. *Biodiversity and Conservation*, **6**, 495–514.

Keller C., Roos C., Groeneveld L.F., Fischer J., & Zinner D. (2010) Introgressive hybridization in southern African baboons shapes patterns of mtDNA variation. *American Journal of Physical Anthropology*, **142**, 125–136.

Kimura M. & Weiss G.H. (1964) The stepping-stone model of population structure and the decrease of genetic correlation with distance. *Genetics*, **49**, 561–576.

Kingdon J. (1984) *East African mammals: an atlas of evolution in Africa.* University of Chicago Press, Chicago.

Kingdon J. (1997) *The Kingdon fieldguide to African mammals.* Academic Press, London.

Kingdon J., Butynski T.M. (2008) *Chlorocebus aethiops*. *IUCN Red List of Threatened Species.* Version 2012.1. Available at <www.iucnredlist.org>. Downloaded on 03 August 2012.

Kingdon J., Gippoliti S. (2008a). *Chlorocebus sabaeus*. *IUCN Red List of Threatened Species.* Version 2012.1. Available at <www.iucnredlist.org>. Downloaded on 03 August 2012.

Kingdon J., Gippoliti S. (2008b). *Chlorocebus tantalus*. *IUCN Red List of Threatened Species.* Version 2012.1. Available at <www.iucnredlist.org>. Downloaded on 03 August 2012.

Kingdon J, Gippoliti S, Butynski TM, de Jong Y. 2008. *Chlorocebus pygerythrus*. IUCN Red List of Threatened Species. Version 2012.1. Available at <www.iucnredlist.org>. Downloaded on 03 August 2012.

Kotlík P., Deffontaine V., Mascheretti S., Zima J., Michaux J.R., & Searle J.B. (2006) A northern glacial refugium for bank voles (*Clethrionomys glareolus*). *Proceedings of the National Academy of Sciences*, **103**, 14860–14864.

Kubatko L.S. (2009) Identifying hybridization events in the presence of coalescence via model selection. *Systematic Biology*, **58**, 478–488.

Van der Kuyl A.C. (1996) St . Kitts Green Monkeys Originate From West Africa : Genetic Evidence From Feces. *American Journal of Primatology*, **40**, 361–364.

Van der Kuyl A.C., Kuiken C.L., Dekker J.T., & Goudsmit J. (1995) Phylogeny of African Monkeys based upon mitonchondrial 12S rRNA sequences. *Journal of Molecular Evolution*, **40**, 173–180.

Larrasoana J.C., Roberts a. P., Rohling E.J., Winklhofer M., & Wehausen R. (2003) Three million years of monsoon variability over the northern Sahara. *Climate Dynamics*, **21**, 689–698.

Leakey M. (1993) Evolution of *Theropithecus* in the Turkana Basin. *Theropithecus, the Rise and Fall of a Primate Genus* (ed. by N. Jablonski), pp. 85–124. Cambridge University Press, Cambridge.

Lebatard A., Bourlès D., Duringer P., Jolivet M., Braucher R., Carcaillet J., Schuster M., Arnaud N., Monié P., Lihoreau F., Likius A., Mackaye H., Vignaud P., & Brunet M. (2008) Cosmogenic nuclide dating of *Sahelanthropus tchadensis* and *Australopithecus bahrelghazali*: Mio-Pliocene hominids from Chad. *Proceedings of the National Academy of Sciences*, **105**, 3226–3231.

Lernould J. (1988) Classification and geographical distribution of guenons: a review. *A Primate Radiation: Evolutionary Biology of the African Guenons* (ed. by A. Gautier-Hion, F. Bourliere, J. Gautier, and J. Kingdon), pp. 54–78. Cambridge University Press, Cambridge.

Li J., Han K., Xing J., Kim H.-S., Rogers J., Ryder O.A., Disotell T., Yue B., & Batzer M.A. (2009) Phylogeny of the macaques (Cercopithecidae: *Macaca*) based on Alu elements. *Gene*, **448**, 242–249.

Lorenzen E.D., Heller R., & Siegismund H.R. (2012) Comparative phylogeography of African savannah ungulates. *Molecular Ecology*, **21**, 3656–3670.

Lorenzen E.D., Masembe C., Arctander P., & Siegismund H.R. (2010) A long-standing Pleistocene refugium in southern Africa and a mosaic of refugia in East Africa: insights from mtDNA and the common eland antelope. *Journal of Biogeography*, **37**, 571–581.

Lorenzen E.D., De Neergaard R., Arctander P., & Siegismund H.R. (2007) Phylogeography, hybridization and Pleistocene refugia of the kob antelope (*Kobus kob*). *Molecular Ecology*, **16**, 3241–3252.

Lourens L., Hilgen F., Shackleton N.J., Laskar J., & Wilson D. (2004) The Neogene period. *A Geologic Time Scale* (ed. by F. Gradstein, J.G. Ogg, and G. Smith), pp. 409–440. Cambridge University Press,

Lukoschek V., Scott Keogh J., & Avise J.C. (2012) Evaluating fossil calibrations for dating phylogenies in light of rates of molecular evolution: a comparison of three approaches. *Systematic Biology*, **61**, 22–43.

Mallet J. (2005) Hybridization as an invasion of the genome. *Trends In Ecology & Evolution*, **20**, 229–237.

Mallet J. (2007) Hybrid speciation. *Nature*, **446**, 279–283.

Markolf M., Brameier M., & Kappeler P.M. (2011) On species delimitation: yet another lemur species or just genetic variation? *BMC Evolutionary Biology*, **11**, e216.

Matthee C. a & Davis S.K. (2001) Molecular insights into the evolution of the family Bovidae: a nuclear DNA perspective. *Molecular Biology and Evolution*, **18**, 1220–1230.

Matthee C. a & Robinson T.J. (1999) Cytochrome b phylogeny of the family bovidae: resolution within the alcelaphini, antilopini, neotragini, and tragelaphini. *Molecular Phylogenetics and Evolution*, **12**, 31–46.

Mayr E. (1963) *Animal Species and Evolution.* Belknap Press, Cambridge.

Mekonnen A., Bekele A., Fashing P.J., Hemson G., & Atickem A. (2010a) Diet, Activity Patterns, and Ranging Ecology of the Bale Monkey (*Chlorocebus djamdjamensis*) in Odobullu Forest, Ethiopia. *International Journal of Primatology*, **31**, 339–362.

Mekonnen A., Bekele A., Fashing P.J., Lernould J.-M., Atickem A., & Stenseth N.C. (2012) Newly discovered Bale monkey populations in forest fragments in southern Ethiopia: evidence of crop raiding, hybridization with grivets, and other conservation threats. *American Journal of Primatology*, **74**, 423–432.

Mekonnen A., Bekele A., Hemson G., Teshome E., & Atickem A. (2010b) Population size and habitat preference of the Vulnerable Bale monkey *Chlorocebus djamdjamensis* in Odobullu Forest and its distribution across the Bale Mountains, Ethiopia. *Oryx*, **44**, 558–563.

Meyer M., Kircher M., Gansauge M.-T., Li H., Racimo F., Mallick S., Schraiber J.G., Jay F., Prüfer K., De Filippo C., Sudmant P.H., Alkan C., Fu Q., Do R., Rohland N., Tandon A., Siebauer M., Green R.E., Bryc K., Briggs A.W., Stenzel U., Dabney J., Shendure J., Kitzman J., Hammer M.F., Shunkov M. V, Derevianko A.P., Patterson N., Andrés A.M., Eichler E.E., Slatkin M., Reich D., Kelso J., & Pääbo S. (2012) A high-coverage genome sequence from an archaic Denisovan individual. *Science*, **338**, 222–226.

Morgan K., O'Loughlin S.M., Chen B., Linton Y.-M., Thongwat D., Somboon P., Fong M.Y., Butlin R., Verity R., Prakash A., Htun P.T., Hlaing T., Nambanya S., Socheat D., Dinh T.H., & Walton C. (2011) Comparative phylogeography reveals a shared impact of pleistocene environmental change in shaping genetic diversity within nine Anopheles mosquito species across the Indo-Burma biodiversity hotspot. *Molecular Ecology*, **20**, 4533–4549.

Moritz C., Hoskin C.J., MacKenzie J.B., Phillips B.L., Tonione M., Silva N., VanDerWal J., Williams S.E., & Graham C.H. (2009) Identification and dynamics of a cryptic suture zone in tropical rainforest. *Proceedings of the Royal Society London B*, **276**, 1235–1244.

Muwanika V.B., Nyakaana S., Siegismund H.R., & Arctander P. (2003) Phylogeography and population structure of the common warthog (*Phacochoerus africanus*)

inferred from variation in mitochondrial DNA sequences and microsatellite loci. *Heredity*, **91**, 361–372.

Müller M.C. & Barré-Sinoussi F. (2003) SIVagm: genetic and biological features associated with replication. *Frontiers in Bioscience*, **8**, 1170–1185.

Naidu A., Fitak R.R., Munguia-Vega A., & Culver M. (2012) Novel primers for complete mitochondrial cytochrome b gene sequencing in mammals. *Molecular Ecology Resources*, **12**, 191–196.

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.* British Museum (Natural History), London.

Newman T.K., Jolly C.J., & Rogers J. (2004) Mitochondrial phylogeny and systematics of baboons (*Papio*). *American Journal of Physical Anthropology*, **124**, 17–27.

Nichol J.E. (1999) Geomorphological evidence and Pleistocene refugia in Africa. *The Geographical Journal*, **165**, 79–89.

Nsubuga A.M., Robbins M.M., Roeder A.D., Morin P.A., Boesch C., & Vigilant L. (2004) Factors affecting the amount of genomic DNA extracted from ape faeces and the identification of an improved sample storage method. *Molecular Ecology*, **13**, 2089–2094.

Nyakaana S., Arctander P., & Siegismund H.R. (2002) Population structure of the African savannah elephant inferred from mitochondrial control region sequences and nuclear microsatellite loci. *Heredity*, **89**, 90–8.

Odee D.W., Telford a, Wilson J., Gaye a, & Cavers S. (2012) Plio-Pleistocene history and phylogeography of Acacia senegal in dry woodlands and savannahs of sub-Saharan tropical Africa: evidence of early colonisation and recent range expansion. *Heredity*, **109**, 372–382.

Orr H.A. (1997) Haldane's Rule. *Annual Review of Ecology and Systematics*, **28**, 195–218.

Pennington R.T., Prado D.E., & Pendry C.A. (2000) Neotropical seasonally dry forests and Quaternary vegetation changes. *Journal of Biogeography*, **27**, 261–273.

Perelman P., Johnson W.E., Roos C., Seuánez H.N., Horvath J.E., Moreira M. a M., Kessing B., Pontius J., Roelke M., Rumpler Y., Schneider M.P.C., Silva A., O'Brien S.J., & Pecon-Slattery J. (2011) A molecular phylogeny of living primates. *PLoS Genetics*, **7**, e1001342.

Pitra C., Kock R.A., Hofmann R., & Lieckfeldt D. (1998) Molecular phylogeny of the critically endangered Hunter's antelope (*Beatragus hunteri* Sclater 1889). *Journal of Zoological Systematics and Evolutionary Research*, **36**, 179–184.

Plana V. (2004) Mechanisms and tempo of evolution in the African Guineo-Congolian rainforest. *Philosophical Transactions of the Royal Society of London B*, **359**, 1585–1594.

Pons J.-M., Olioso G., Cruaud C., & Fuchs J. (2011) Phylogeography of the Eurasian green woodpecker (*Picus viridis*). *Journal of Biogeography*, **38**, 311–325.

Posada D. (2008) jModelTest: phylogenetic model averaging. *Molecular Biology and Evolution*, **25**, 1253–1256.

Potts R. (1998) Environmental hypotheses of hominin evolution. *Yearbook of Physical Anthropology*, **41**, 93–136.

Potts R. (2012) Environmental and Behavioral Evidence Pertaining to the Evolution of Early Homo. *Current Anthropology*, **53**, S299–S317.

Pozzi L., Hodgson J., Burrell A., & Disotell T. (2011) The stem catarrhine *Saadanius* does not inform the timing of the origin of crown catarrhines. *Journal of Human Evolution*, **61**, 209–210.

Rambaut A. & Drummond A.J. (2007) *Tracer v1.4*. Available at http://beast.bio.ed.ac.uk/Tracer%20.

Roberts T.E., Davenport T.R.B., Hildebrandt K.B.P., Jones T., Stanley W.T., Sargis E.J., & Olson L.E. (2010) The biogeography of introgression in the critically endangered African monkey *Rungwecebus kipunji*. *Biology Letters*, **6**, 233–237.

Rohland N. & Hofreiter M. (2007) Ancient DNA extraction from bones and teeth. *Nature Protocols*, **2**, 1756–1762.

Rohland N., Siedel H., & Hofreiter M. (2004) Nondestructive DNA extraction method for mitochondrial DNA analyses of museum specimens. *BioTechniques*, **36**, 814–821.

Rohland N., Siedel H., & Hofreiter M. (2010) A rapid column-based ancient DNA extraction method for increased sample throughput. *Molecular Ecology Resources*, **10**, 677–683.

Ronquist F. (1997) Dispersal-vicariance analysis: a new approach to the quantification of historical biogeography. *Systematic Biology*, **46**, 195-203.

Ronquist F. & Huelsenbeck J. (2003) MRBAYES3: Bayesian phylogenetic inference under mixed models. *Bioinformatics*, **19**, 1572–1574.

Roos C., Nadler T., & Walter L. (2008) Mitochondrial phylogeny, taxonomy and biogeography of the silvered langur species group (*Trachypithecus cristatus*). *Molecular Phylogenetics and Evolution*, **47**, 629–636.

Roos C., Zinner D., Kubatko L.S., Schwarz C., Yang M., Meyer D., Nash S.D., Xing J., Batzer M. a, Brameier M., Leendertz F.H., Ziegler T., Perwitasari-Farajallah D., Nadler T., Walter L., & Osterholz M. (2011) Nuclear versus mitochondrial DNA: evidence for hybridization in colobine monkeys. *BMC Evolutionary Biology*, **11**, e77.

Ropiquet A. & Hassanin A. (2005) Molecular phylogeny of caprines (Bovidae, Antilopinae): the question of their origin and diversification during the Miocene. *Journal of Zoological Systematics and Evolutionary Research*, **43**, 49–60.

Rundle H.D. & Nosil P. (2005) Ecological speciation. *Ecology Letters*, **8**, 336–352.

Schilthuizen M., Giesbers M.C.W.G., & Beukeboom L.W. (2011) Haldane's rule in the 21st century. *Heredity*, **107**, 95–102.

Scholz C. a, Johnson T.C., Cohen A.S., King J.W., Peck J. a, Overpeck J.T., Talbot M.R., Brown E.T., Kalindekafe L., Amoako P.Y.O., Lyons R.P., Shanahan T.M., Castañeda I.S., Heil C.W., Forman S.L., McHargue L.R., Beuning K.R., Gomez J., & Pierson J. (2007) East African megadroughts between 135 and 75 thousand years ago and bearing on early-modern human origins. *Proceedings of the National Academy of Sciences*, **104**, 16416–16421.

Schwarz E. (1926) Die Meerkatzen der *Cercopithecus aethiops*-Gruppe. *Zeitschrift für Säugetierkunde*, **1**, 28–47.

Schönswetter P., Stehlik I., Holderegger R., & Tribsch a (2005) Molecular evidence for glacial refugia of mountain plants in the European Alps. *Molecular Ecology*, **14**, 3547–3555.

Seehausen O. (2013) Conditions when hybridization might predispose populations for adaptive radiation. *Journal of Evolutionary Biology*, **26**, 279-281.

Seehausen O., Takimoto G., Roy D., & Jokela J. (2008) Speciation reversal and biodiversity dynamics with hybridization in changing environments. *Molecular Ecology*, **17**, 30–44.

Shapiro B., Drummond A.J., Rambaut A., Wilson M.C., Matheus P.E., Sher A. V, Pybus O.G., Gilbert M.T.P., Barnes I., Binladen J., Willerslev E., Hansen A.J., Baryshnikov G.F., Burns J. a, Davydov S., Driver J.C., Froese D.G., Harington C.R., Keddie G., Kosintsev P., Kunz M.L., Martin L.D., Stephenson R.O., Storer J., Tedford R., Zimov S., & Cooper A. (2004) Rise and fall of the Beringian steppe bison. *Science*, **306**, 1561–1565.

Shimada M.K. (2000) Geographic distribution of mitochondrial DNA variations among grivet (*Cercopithecus aethiops aethiops*) populations in Central Ethiopia. *International Journal of Primatology*, **21**, 113–129.

Shimada M.K. & Shotake T. (1997) Genetic variation of blood proteins within and between local populations of grivet monkey (*Cercopithecus aethiops aethiops*) in Central Ethiopia. *Primates*, **38**, 399–414.

Shimada M.K., Terao K., & Shotake T. (2002) Mitochondrial sequence diversity within a subspecies of savanna monkeys (*Cercopithecus aethiops*) is similar to that between subspecies. *The Journal of Heredity*, **93**, 9–18.

Smitz N., Berthouly C., Cornélis D., Heller R., Van Hooft P., Chardonnet P., Caron A., Prins H., Van Vuuren B.J., De Iongh H., & Michaux J. (2013) Pan-African genetic structure in the African Buffalo (*Syncerus caffer*): investigating intraspecific divergence. *PloS ONE*, **8**, e56235.

Stemshorn K.C., Reed F. a, Nolte A.W., & Tautz D. (2011) Rapid formation of distinct hybrid lineages after secondary contact of two fish species (*Cottus* sp.). *Molecular Ecology*, **20**, 1475–1491.

Stewart J.R., Lister A.M., Barnes I., & Dalén L. (2010) Refugia revisited: individualistic responses of species in space and time. *Proceedings of the Royal Society B*, **277**, 661–671.

Switzer W., Salemi M., Shanmugam V., Cong M., Kuiken C., Bhullar V., Beer B.E., Vallet D., Gautier-Hion A., Tooze Z., Villinger F., Holmes E.C., & Heneine W. (2005) Ancient co-speciation of simian foamy viruses and primates. *Nature*, **434**, 376–380.

Swofford D. (2003) *PAUP*. Phylogenetic analysis using parsimony (*and other methods).* Sinauer Associates, Sunderland (MA).

Taberlet P., Fumagalli L., Wust-Saucy A.-G., & Cosson J.F. (1998) Comparative phylogeography and postglacial colonization. *Molecular Ecology*, **7**, 453–464.

Talavera G. & Castresana J. (2007) Improvement of phylogenies after removing divergent and ambiguously aligned blocks from protein sequence alignments. *Systematic Biology*, **56**, 564–577.

Tamura K., Peterson D., Peterson N., Stecher G., Nei M., & Kumar S. (2011) MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. *Molecular Biology and Evolution*, **28**, 2731–2739.

Thinh V.N., Rawson B., Hallam C., Kenyon M., Nadler T., Walter L., & Roos C. (2010) Phylogeny and distribution of crested gibbons (genus *Nomascus*) based on mitochondrial cytochrome b gene sequence data. *American Journal of Primatology*, **72**, 1047–1054.

Tobe S.S., Kitchener A.C., & Linacre A.M.T. (2010) Reconstructing mammalian phylogenies: a detailed comparison of the cytochrome B and cytochrome oxidase subunit I mitochondrial genes. *PloS ONE*, **5**, e14156.

Toit J.T. Du & Cumming D.H.M. (1999) Functional significance of ungulate diversity in African savannas and the ecological implications of the spread of pastoralism. *Biodiversity and Conservation*, **8**, 1643–1661.

Tosi A.J., Morales J.C., & Melnick D.J. (2002) Y-chromosome and mitochondrial markers in *Macaca fascicularis* indicate introgression with Indochinese *M. mulatta* and a biogeographic barrier in the Isthmus of Kra. *International Journal of Primatology*, **23**, 161–178.

Tosi A.J., Morales J.C., & Melnick D.J. (2003) Paternal, maternal, and biparental molecular markers provide unique windows onto the evolutionary history of macaque monkeys. *Evolution*, **57**, 1419–1435.

Trauth M.H., Larrasoaña J.C., & Mudelsee M. (2009) Trends, rhythms and events in Plio-Pleistocene African climate. *Quaternary Science Reviews*, **28**, 399–411.

Trauth M.H., Maslin M.A., Deino A., Junginger A., Lesoloyia M., & Eric O. (2010) Human evolution in a variable environment : the amplifier lakes of Eastern Africa. *Quaternary Science Reviews*, **29**, 2981–2988.

Trauth M.H., Maslin M.A., Deino A., & Strecker M.R. (2005) Late Cenozoic moisture history of East Africa. *Science*, **309**, 2051–3.

Trauth M.H., Maslin M.A., Deino A.L., Strecker M.R., Bergner A.G.N., & Dühnforth M. (2007) High- and low-latitude forcing of Plio-Pleistocene East African climate and human evolution. *Journal of Human Evolution*, **53**, 475–86.

Tung J., Charpentier M.J.E., Garfield D. a, Altmann J., & Alberts S.C. (2008) Genetic evidence reveals temporal change in hybridization patterns in a wild baboon population. *Molecular ecology*, **17**, 1998–2011.

Tzedakis C. (2003) Timing and duration of Last Interglacial conditions in Europe: a chronicle of a changing chronology. *Quaternary Science Reviews*, **22**, 763–768.

Vignaud P., Duringer P., Mackaye H., Likius A., Blondel C., Boisserie J., De Bonis L., Eisenmann V., Etienne M., Geraads D., Guy F., Lehmann T., Lihoreau F., Lopez-Martinez N., Mourer-Chauviré C., Otero O., Rage J., Schuster M., Viriot L., Zazzo A., & Brunet M. (2002) Geology and palaeontology of the Upper Miocene Toros-Menalla hominid locality, Chad. *Nature*, **418**, 152–155.

Vrba E.S. (1979) Phylogenetic analysis and classification of fossil and recent Alcelaphini Mammalia: Bovidae. *Biological Journal of the Linnean Society*, **11**, 207–228.

Vrba E.S. (1999) Habitat theory in relation to the evolution in African neogene biota and hominids. *African Biogeography, Climate Change, & Human Evolution* (ed. by T. Bromage and F. Schrenk), pp. 19–34. Oxford University Press, Oxford.

Wertheim J.O. & Worobey M. (2007) A challenge to the ancient origin of SIVagm based on African green monkey mitochondrial genomes. *PLoS Pathogens*, **3**, e95.

Wildman D.E., Bergman T.J., Al-Aghbari A., Sterner K.N., Newman T.K., Phillips-Conroy J.E., Jolly C.J., & Disotell T.R. (2004) Mitochondrial evidence for the origin of hamadryas baboons. *Molecular Phylogenetics and Evolution*, **32**, 287–296.

Wolf D.E., Takebayashi N., & Rieseberg L.H. (2001) Predicting the Risk of Extinction through Hybridization. *Conservation Biology*, **15**, 1039–1053.

Xing J., Wang H., Zhang Y., Ray D. a, Tosi A.J., Disotell T.R., & Batzer M. a (2007) A mobile element-based evolutionary history of guenons (tribe Cercopithecini). *BMC Biology*, **5**, e5.

Yokoyama Y., Lambeck K., De Deckker P, Johnston P., & Fifield L. (2000) Timing of the Last Glacial Maximum from observed sea-level minima. *Nature*, **406**, 713–716.

Zachos F.E., Apollonio M., Bärmann E. V., Festa-Bianchet M., Göhlich U., Habel J.C., Haring E., Kruckenhauser L., Lovari S., McDevitt A.D., Pertoldi C., Rössner G.E., Sánchez-Villagra M.R., Scandura M., & Suchentrunk F. (2012) Species inflation and taxonomic artefacts — A critical comment on recent trends in mammalian classification. *Mammalian Biology*, **78**, 1–6.

Zachos J., Pagani M., Sloan L., Thomas E., & Billups K. (2001) Trends, rhythms, and aberrations in global climate 65 Ma to present. *Science*, **292**, 686–693.

Zalmout I.S., Sanders W.J., Maclatchy L.M., Gunnell G.F., Al-Mufarreh Y. a, Ali M.A, Nasser A.-A.H., Al-Masari A.M., Al-Sobhi S. a, Nadhra A.O., Matari A.H., Wilson J.A, & Gingerich P.D. (2010) New Oligocene primate from Saudi Arabia and the divergence of apes and Old World monkeys. *Nature*, **466**, 360–364.

Zinner D., Arnold M.L., & Roos C. (2009a) Is the new primate genus *Rungwecebus* a baboon? *PloS one*, **4**, e4859.

Zinner D., Arnold M.L., & Roos C. (2011a) The strange blood: natural hybridization in primates. *Evolutionary Anthropology*, **20**, 96–103.

Zinner D., Buba U., Nash S., & Roos C. (2011b) Pan-African voyagers: the phylogeography of baboons. *Primates of Gashaka, Developments in Primatology: Progress and Prospects* (ed. by V. Sommer and C. Ross), pp. 267–306. Springer New York, New York.

Zinner D., Groeneveld L.F., Keller C., & Roos C. (2009b) Mitochondrial phylogeography of baboons (*Papio* spp.): indication for introgressive hybridization? *BMC Evolutionary Biology*, **9**, 83.

Zinner D. & Roos C. (2010) Einfluss von Hybridisierung in der Evolution von Primaten Impact of hybridization in primate evolution. *Braunschweiger Naturkundliche Schriften*, **9**, 135–156.

Zinner D., Wertheimer J., Liedigk R., Groeneveld L., & Roos C. (2013) Baboon phylogeny as inferred from complete mitochondrial genomes. *American Journal of Physical Anthropology*, **150**, 133–140.

Zwickl D. (2006) *Genetic algorithm approaches for the phylogenetic analysis of large biological sequence datasets under the maximum likelihood criterion.* Ph.D. thesis, Univeristy of Texas, Austin, TX.

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APPENDIX I

Supplementary material of Chapter 4:

Table S4.I Sample information and GenBank accession numbers of sequences used in this study. GenBank accession numbers of the first and second column indicate sequences used for demographic and phylogenetic analyses, respectively. Cyt *b*=cytochrome *b*, RSA=Republic of South Africa, CAR=Central African Republic, DRC=Democratic Republic of the Congo.

ID	Taxon	Country	GenBank accession no.	
Chlorocebus [1]			**cyt *b***	**Brown**
I	*pygerythrus*	RSA	EF597500	x
II	*pygerythrus*	Tanzania /Kenya	EF597501	x
III	*tantalus*	CAR	EF597502	x
IV	*sabaeus*	Senegal	EF597503	x
1	*aethiops*	Eritrea	JX983734	-
2	*aethiops*	Eritrea	JX983735	-
3	*aethiops*	Eritrea	JX983733	-
8	*aethiops*	Eritrea	JX983730	-
9	*aethiops*	Eritrea	JX983731	-
11	*aethiops*	Eritrea	JX983732	-
26	*tantalus*	CAR	JX983846	-
64	*pygerythrus*	RSA	JX983769	x
65	*pygerythrus*	RSA	JX983770	-
70	*cynosuros*	Zambia	JX983756	-
72	*pygerythrus*	Zambia	JX983752	x
75	*pygerythrus*	RSA	JX983771	-
78	*pygerythrus*	RSA	JX983772	-
91	*cynosuros*	Zambia	JX983755	x
101	*cynosuros*	Zambia	JX983754	x
102	*tantalus*	Nigeria	JX983843	x
103	*tantalus*	Nigeria	JX983844	-
104	*tantalus*	Nigeria	JX983845	x
118	*sabaeus*	Senegal	JX983827	x
120	*sabaeus*	Senegal	JX983828	-
138	*sabaeus*	Ghana	JX983804	-
139	*sabaeus*	Ghana	JX983805	x
142	*sabaeus*	Ghana	JX983806	-
143	*sabaeus*	Ghana	JX983807	-
144	*sabaeus*	Ghana	JX983808	-
145	*sabaeus*	Ghana	JX983809	-
146	*sabaeus*	Ghana	JX983810	-
153	*sabaeus*	Ghana	JX983811	-
158	*sabaeus*	Ghana	JX983812	-
160	*sabaeus*	Ghana	JX983813	-

ID	Taxon	Country	GenBank accession no.	
Chlorocebus[1]			**cyt *b***	**Brown**
161	*sabaeus*	Ghana	JX983814	-
165	*sabaeus*	Ghana	JX983815	-
166	*sabaeus*	Ghana	JX983816	-
167	*sabaeus*	Ghana	JX983817	-
168	*sabaeus*	Ghana	JX983818	-
169	*sabaeus*	Ghana	JX983819	-
170	*sabaeus*	Ghana	JX983820	-
171	*sabaeus*	Ghana	JX983821	-
172	*sabaeus*	Ghana	JX983822	-
191	*tantalus*	Ghana	JX983839	x
192	*tantalus*	Ghana	JX983840	x
193	*tantalus*	Ghana	JX983841	-
203	*tantalus*	Ghana	JX983823	x
208	*tantalus*	Ghana	JX983824	-
212	*tantalus*	Burkina Faso	JX983836	-
213	*tantalus*	Burkina Faso	JX983837	-
215	*tantalus*	Burkina Faso	JX983838	-
223	*tantalus*	Burkina Faso	JX983830	x
224	*tantalus*	Burkina Faso	JX983831	-
225	*tantalus*	Burkina Faso	JX983832	-
226	*tantalus*	Burkina Faso	JX983833	-
227	*tantalus*	Burkina Faso	JX983834	-
228	*tantalus*	Burkina Faso	JX983835	-
233	*tantalus*	Burkina Faso	JX983829	-
239	*sabaeus*	Burkina Faso	JX983793	-
241	*sabaeus*	Burkina Faso	JX983794	-
246	*sabaeus*	Burkina Faso	JX983795	-
247	*sabaeus*	Burkina Faso	JX983796	x
248	*sabaeus*	Burkina Faso	JX983797	-
250	*sabaeus*	Burkina Faso	JX983798	-
251	*sabaeus*	Burkina Faso	JX983799	-
254	*sabaeus*	Burkina Faso	JX983800	-
255	*sabaeus*	Burkina Faso	JX983801	-
256	*sabaeus*	Burkina Faso	JX983802	-
264	*sabaeus*	Burkina Faso	JX983792	-
268	*sabaeus*	Burkina Faso	JX983778	x
269	*sabaeus*	Burkina Faso	JX983779	-
271	*sabaeus*	Burkina Faso	JX983780	-
272	*sabaeus*	Burkina Faso	JX983781	-
273	*sabaeus*	Burkina Faso	JX983782	-
275	*sabaeus*	Burkina Faso	JX983783	-
277	*sabaeus*	Burkina Faso	JX983784	-
279	*sabaeus*	Burkina Faso	JX983785	-
281	*sabaeus*	Burkina Faso	JX983786	-

ID	Taxon	Country	GenBank accession no.	
Chlorocebus[1]			**cyt *b***	**Brown**
283	*sabaeus*	Burkina Faso	JX983787	-
284	*sabaeus*	Burkina Faso	JX983788	-
285	*sabaeus*	Burkina Faso	JX983789	-
286	*sabaeus*	Burkina Faso	JX983790	-
293	*sabaeus*	Burkina Faso	JX983791	-
316	*aethiops*	Ethiopia	JX983749	x
317	*aethiops*	Ethiopia	JX983750	x
319	*djamdjamensis*	Ethiopia	JX983758	x
320	*djamdjamensis*	Ethiopia	JX983759	-
323	*djamdjamensis*	Ethiopia	JX983760	x
330	*aethiops*	Ethiopia	JX983741	x
331	*aethiops*	Ethiopia	JX983742	-
373	*aethiops*	Ethiopia	JX983740	x
391	*aethiops/pygerythrus*	Ethiopia	JX983739	x
400	*aethiops*	Ethiopia	JX983746	-
421	*pygerythrus*	Ethiopia	JX983766	x
433	*djamdjamensis/aethiops*	Ethiopia	JX983761	-
436	*djamdjamensis/aethiops*	Ethiopia	JX983762	x
438	*aethiops*	Ethiopia	JX983744	-
439	*aethiops*	Ethiopia	JX983745	x
451	*aethiops*	Ethiopia	JX983743	x
464	*aethiops*	Ethiopia	JX983751	x
484	*aethiops*	Ethiopia	JX983747	-
485	*aethiops*	Ethiopia	JX983748	-
496	*pygerythrus*	Ethiopia	JX983764	x
503	*pygerythrus*	Tanzania	JX983773	x
504	*pygerythrus*	Kenya	JX983767	-
507	*pygerythrus*	Kenya	JX983851	-
513	*pygerythrus*	Kenya	JX983850	-
520	*sabaeus*	Liberia	JX983825	-
522	*pygerythrus*	Pemba Island	JX983774	-
524	*pygerythrus*	Tanzania	JX983775	-
528	*aethiops*	Ethiopia	JX983736	-
529	*djamdjamensis*	Ethiopia	JX983757	-
530	*aethiops*	Ethiopia	JX983737	-
535	*sabaeus*	Mauretania	JX983826	-
536	*tantalus*	Togo	JX983842	-
539	*tantalus*	Cameroon	JX983848	-
540	*pygerythrus*	Kenya	JX983768	-
545	*pygerythrus*	Somalia	JX983765	-
546	*cf. pygerythrus*	Ethiopia	JX983763	-
549	*pygerythrus*	Ethiopia	JX983776	-
553	*sabaeus*	Ghana	JX983803	-
560	*pygerythrus*	Uganda	JX983849	-

ID	Taxon	Country	GenBank accession no.	
Chlorocebus[1]			**cyt *b***	**Brown**
561	*tantalus*	Cameroon	JX983847	-
563	*pygerythrus*	Tanzania	JX983777	-
566	*cynosuros*	Angola	JX983753	-
567	*aethiops*	Ethiopia	JX983738	-
Chlorocebus - outgroups				
Epat	*Erythrocebus patas*		x	x
414PHBuH	*Papio hamadryas*		EU885458	EU885805
Cgue	*Colobus guereza*		NC006901	NC006901
Tgel	*Theropithecus gelada*		EU885	EU885
Mmul	*Macaca mulatta*		AY612638	AY612638
Msyl	*Macaca sylvanus*		AJ309865	AJ309865
Ppyg	*Pongo pygmaeus*		NC001646	NC001646
Ptro	*Pan troglodytes*		D38113	D38113
Hsap	*Homo sapiens*		AY339522	AY339522
Papio[2,3]			**cyt *b***	**Brown**
037PHAbd	*hamadryas*	Eritrea	EU885453	
096PAKoN	*anubis*	Côte d'Ivoire	EU885420	EU885767
1192PUMad	*ursinus*	RSA	GQ398081	-
1432PUGra	*ursinus*	RSA	GQ398065	-
1444PUZeb	*ursinus*	RSA	GQ398071	-
1445PUMZN	*ursinus*	RSA	GQ398072	-
1475PUBeu	*ursinus*	RSA	GQ398068	-
1488PUSou	*ursinus*	RSA	GQ398074	-
151PCMic	*cynocephalus*	Malawi	EU885445	EU885792
1537PPSde	*papio*	Mauretania	x	-
1540PPANg	*papio*	Mauretania	x	-
1552PAKwa	*anubis*	Nigeria	x	-
1561PAGas	*anubis*	Nigeria	x	-
1567PAMle	*anubis*	Ghana	x	-
1568PAShH	*anubis*	Ghana	x	-
1569PABR1	*anubis*	Burkina Faso	x	-
1571PACdA	*anubis*	Burkina Faso	x	-
1572PACdA	*anubis*	Burkina Faso	x	-
1573PARNz	*anubis*	Burkina Faso	x	-
184PAGri	*anubis*	Eritrea	EU885434	-
194PAHad	*anubis*	Eritrea	EU885435	-
215PhMen	*hamadryas*	Eritrea	x	-
236PhFil	*hamadryas*	Eritrea	x	-
252PPBak	*papio*	Guinea	EU885463	EU885810
288PCMu2	*cynocephalus*	Malawi	EU885446	-
301PHASt	*hamadryas*	Ethiopia	EU885456	-
319PHGer	*hamadryas*	Ethiopia	EU885457	-

ID	Taxon	Country	GenBank accession no.	
***Papio*[2,3]**			**cyt *b***	**Brown**
340PAWen	*anubis*	Ethiopia	x	-
341PAWen	*anubis*	Ethiopia	x	-
343PAWen	*anubis*	Ethiopia	x	-
349PAMan	*anubis*	Ethiopia	EU885436	-
353PASwr	*anubis*	Tanzania	EU885439	EU885786
391PHAfb	*hamadryas*	Eritrea	EU885455	-
404PCRuk	*cynocephalus*	Tanzania	x	-
409PCLuS	*cynocephalus*	Zambia	EU885447	-
411PCLuS	*cynocephalus*	Zambia	EU885448	-
414PHBuH	*hamadryas*	Yemen	EU885458	EU885805
422PUPil	*ursinus*	RSA	EU885470	-
435PUBly	*ursinus*	RSA	EU885473	-
448PAKib	*anubis*	Uganda	EU885432	EU885779
463PUHop	*ursinus*	RSA	EU885486	EU885833
468PULos	*ursinus*	RSA	EU885478	-
470PULos	*ursinus*	RSA	EU885477	-
472PUHak	*ursinus*	Namibia	EU885480	-
482PuSpr	*ursinus*	Namibia	EU885481	-
492PUMor	*ursinus*	Botswana	EU885469	EU885816
501PUNya	*ursinus*	Zimbabwe	EU885466	-
507PCWeb	*cynocephalus*	Somalia	EU885440	-
510PPZoo	*papio*	Côte d'Ivoire	x	-
512PAsBu	*anubis*	DRC	EU885433	-
518PPKed	*papio*	Senegal	EU885461	-
523PPKed	*papio*	Senegal	EU885462	EU885809
526PPAss	*papio*	Senegal	EU885459	-
529PCDia	*cynocephalus*	Kenya	EU885441	EU885788
537PCAmb	*cynocephalus*	Kenya	EU885443	-
539PUGor	*ursinus*	Mozambique	EU885467	-
541PUDra	*ursinus*	RSA	EU885482	EU885829
545PABwa	*anubis*	Nigeria	EU885421	-
546PALum	*anubis*	Nigeria	EU885422	-
547PAKem	*anubis*	Nigeria	EU885424	EU885771
548PASep	*anubis*	Nigeria	EU885425	-
549PAChi	*anubis*	Nigeria	EU885428	EU885775
550PALum	*anubis*	Nigeria	EU885423	EU885770
552PAKur	*anubis*	Nigeria	EU885430	EU885777
558PUOka	*ursinus*	Namibia	EU885476	-
559PACMR	*anubis*	Cameroon	EU885431	-
560PULub	*ursinus*	Angola	EU885479	-
561PUGoe	*ursinus*	RSA	EU885485	-
563PUIta	*ursinus*	RSA	EU885474	EU885821
565PKKfN	*kindae*	Zambia	EU885450	EU885797
566PUKfM	*ursinus*	Zambia	EU885464	-

ID	Taxon	Country	GenBank accession no.	
Papio[2,3]			**cyt *b***	**Brown**
568PKKas	*kindae*	Zambia	EU885451	-
569PKShi	*kindae*	Zambia	EU885452	EU885452
570PCLua	*cynocephalus*	Zambia	EU885451	-
626PuBri	*ursinus*	Zambia	GQ148683	-
641PKKN1	*kindae*	Zambia	GQ148708	-
658PKKai	*kindae*	Zambia	GQ148711	-
705PUKm1	*ursinus*	Namibia	GQ148675	-
710PUOj1	*ursinus*	Namibia	GQ148671	-
727PUnWh	*ursinus*	Namibia	GQ148663	-
732PUWwi	*ursinus*	Zambia	GQ148691	-
800PUC28	*ursinus*	Namibia	GQ148679	-
819PUKlp	*ursinus*	Namibia	GQ148677	-
835PUwOm	*ursinus*	Namibia	GQ148653	-
904PUSTR	*ursinus*	Namibia	GQ148659	-
Papio - outgroups				
Cgue	*Colobus guereza*	-	NC006901	NC006901
Tgel	*Theropithecus gelada*	-	EU885	EU885
Mmul	*Macaca mulatta*	-	AY612638	AY612638
Msyl	*Macaca sylvanus*	-	AJ309865	AJ309865
Ppyg	*Pongo pygmaeus*	-	NC001646	NC001646
Ptro	*Pan troglodytes*	-	D38113	D38113
Hsap	*Homo sapiens*	-	AY339522	AY339522
Alcelaphus[4-8]			**D-loop**	**cyt *b***
caama721 1079nam	*caama*	Namibia	AF167721	-
caama722 1080nam	*caama*	Namibia	AF167722	-
caama723 1109nam	*caama*	Namibia	AF167723	-
caama724 1122nam	*caama*	Namibia	AF167724	-
caama725 2414nam	*caama*	Namibia	AF167725	-
lelwel726 1202ug	*lelwel*	Uganda	AF167726	-
lelwel728 1214ug	*lelwel*	Uganda	AF167728	-
lelwel730 4757ug	*lelwel*	Uganda	AF167730	-
major731 4765cam	*major*	Cameroon	AF167731	-
major732 4766cam	*major*	Cameroon	AF167732	-
major746 6399gha	*major*	Ghana	AF167734	-
major736 6402gha	*major*	Ghana	AF167735	-
major734 6404gha	*major*	Ghana	AF167736	-
licht BW023016	*lichtensteinii*	Tanzania	AF167737	-
licht738 BW033832	*lichtensteinii*	Tanzania	AF167738	-
licht739 BW044186	*lichtensteinii*	Tanzania	AF167739	-
licht740 BW054187	*lichtensteinii*	Tanzania	AF167740	-
licht741 BW064188	*lichtensteinii*	Tanzania	AF167741	-
licht742 BW075168	*lichtensteinii*	Tanzania	AF167742	-

ID	Taxon	Country	GenBank accession no.	
***Alcelaphus*[4-8]**			**D-loop**	**cyt *b***
licht743 BW085600	*lichtensteinii*	Tanzania	AF167743	-
licht744 BW093512	*lichtensteinii*	Tanzania	AF167744	-
licht745 BW103514	*lichtensteinii*	Tanzania	AF167745	-
major735 6405gha	*major*	Ghana	AF167746	-
cokii748 co3039tz	*cokii*	Tanzania	AF167748	-
caama749 GH011929	*caama*	Botswana	AF167749	-
caama750 GH021954	*caama*	Botswana	AF167750	-
caama752 GH051986	*caama*	Botswana	AF167752	-
caama753 GH062004	*caama*	Botswana	AF167753	-
licht755 IRK04420	*lichtensteinii*	Tanzania	AF167755	-
licht756 IRK05303	*lichtensteinii*	Tanzania	AF167756	-
licht757 IRK06303	*lichtensteinii*	Tanzania	AF167757	-
licht758 IRK07303	*lichtensteinii*	Tanzania	AF167758	-
licht759 IRK08352	*lichtensteinii*	Tanzania	AF167759	-
licht760 IRK09352	*lichtensteinii*	Tanzania	AF167760	-
licht761 IRK10352	*lichtensteinii*	Tanzania	AF167761	-
licht762 IRK12559	*lichtensteinii*	Tanzania	AF167762	-
licht763 IRK13559	*lichtensteinii*	Tanzania	AF167763	-
licht764 IRK14559	*lichtensteinii*	Tanzania	AF167764	-
licht765 IRK15559	*lichtensteinii*	Tanzania	AF167765	-
licht766 IRK17419	*lichtensteinii*	Tanzania	AF167766	-
licht768 IRK20303	*lichtensteinii*	Tanzania	AF167768	-
licht769 KF012473	*lichtensteinii*	Zambia	AF167769	-
licht770 KF024580	*lichtensteinii*	Zambia	AF167770	-
licht771 KF034581	*lichtensteinii*	Zambia	AF167771	-
licht 772KF044582	*lichtensteinii*	Zambia	AF167772	-
licht733 KF054583	*lichtensteinii*	Zambia	AF167773	-
licht734 KF062456	*lichtensteinii*	Zambia	AF167774	-
licht775 KS012605	*lichtensteinii*	Zambia	AF167775	-
licht776 KS022608	*lichtensteinii*	Zambia	AF167776	-
licht777 KS032609	*lichtensteinii*	Zambia	AF167777	-
licht778 KS042610	*lichtensteinii*	Zambia	AF167778	-
licht779 KS052611	*lichtensteinii*	Zambia	AF167779	-
licht780 KS062612	*lichtensteinii*	Zambia	AF167780	-
licht781 KS072613	*lichtensteinii*	Zambia	AF167781	-
licht782 li3036tz	*lichtensteinii*	Tanzania	AF167782	-
cokii783 MK013018	*cokii*	Tanzania	AF167783	-
cokii784 MK023509	*cokii*	Tanzania	AF167784	-
cokii786 MK044193	*cokii*	Tanzania	AF167786	-
cokii787 MK064195	*cokii*	Tanzania	AF167787	-
cokii789 MK105602	*cokii*	Tanzania	AF167789	-
cokii790 MK115603	*cokii*	Tanzania	AF167790	-
cokii791 MK153017	*cokii*	Tanzania	AF167791	-
cokii792 MK163019	*cokii*	Tanzania	AF167792	-

ID	Taxon	Country	GenBank accession no.	
Alcelaphus[4-8]			**D-loop**	**cyt *b***
cokii794 MK205604	*cokii*	Tanzania	AF167794	-
cokii796 MM03610	*cokii*	Kenya	AF167796	-
cokei797 MM05612	*cokii*	Kenya	AF167797	-
cokii798 MM06613	*cokii*	Kenya	AF167798	-
cokeiMM090616	*cokii*	Kenya	AF167799	-
cokii800 MM13620	*cokii*	Kenya	AF167800	-
cokii MM14621	*cokii*	Kenya	AF167801	-
cokii802 MM15622	*cokii*	Kenya	AF167802	-
cokii803 NA01591	*cokii*	Kenya	AF167803	-
cokii804 NA02592	*cokii*	Kenya	AF167804	-
cokii806 NA08598	*cokii*	Kenya	AF167806	-
cokii807 NA09599	*cokii*	Kenya	AF167807	-
cokii808 NA11601	*cokii*	Kenya	AF167808	-
cokii809 NA12602	*cokii*	Kenya	AF167809	-
licht810 SEL01	*lichtensteinii*	Tanzania	AF167810	-
licht811 SEL02	*lichtensteinii*	Tanzania	AF167811	-
licht813 SEL043510	*lichtensteinii*	Tanzania	AF167813	-
swaynei815 SEN023702	*swaynei*	Ethiopia	AF167815	-
licht821 UG013022	*lichtensteinii*	Tanzania	AF167821	-
licht822 UG023026	*lichtensteinii*	Tanzania	AF167822	-
licht824 UG044190	*lichtensteinii*	Tanzania	AF167824	-
licht825 UG105591	*lichtensteinii*	Tanzania	AF167825	-
licht826 UG115592	*lichtensteinii*	Tanzania	AF167826	-
licht827 UG153496	*lichtensteinii*	Tanzania	AF167827	-
licht828 UG194198	*lichtensteinii*	Tanzania	AF167828	-
licht829 UG204199	*lichtensteinii*	Tanzania	AF167829	-
licht830 UG213021	*lichtensteinii*	Tanzania	AF167830	-
licht831 UG223023	*lichtensteinii*	Tanzania	AF167831	-
swaynei1 001-009	*swaynei*	Ethiopia	AF300881	AF300936
swaynei2 010-015	*swaynei*	Ethiopia	AF300882	-
swaynei3 016	*swaynei*	Ethiopia	AF300883	-
swaynei4 017-018	*swaynei*	Ethiopia	AF300884	AF300937
swaynei23 73.169	*swaynei*	Ethiopia	AF300885	-
swaynei5 019	*swaynei*	Ethiopia	AF300886	-
swaynei6 020	*swaynei*	Ethiopia	AF300887	-
swaynei7 021	*swaynei*	Ethiopia	AF300888	-
swaynei8 022	*swaynei*	Ethiopia	AF300889	-
swaynei9 023	*swaynei*	Ethiopia	AF300890	-
swaynei10 024-025	*swaynei*	Ethiopia	AF300891	AF300938
swaynei11 026-040	*swaynei*	Ethiopia	AF300892	-
swaynei12 041	*swaynei*	Ethiopia	AF300893	-
swaynei13 042-043	*swaynei*	Ethiopia	AF300894	-
swaynei14 044	*swaynei*	Ethiopia	AF300895	-

ID	Taxon	Country	GenBank accession no.	
***Alcelaphus*[4-8]**			**D-loop**	**cyt *b***
swaynei45 11.1.54	*swaynei*	Ethiopia	AF300896	-
swaynei68 AbysII65	*swaynei*	Ethiopia	AF300897	-
somali 93.12.1.6	*swaynei*	Somalia	AF300898	-
caama4 004	*caama*	Namibia	AF300899	-
caama5 005	*caama*	Namibia	AF300900	-
caama9 009	*caama*	Namibia	AF300901	-
licht59 Tang7	*lichtensteinii*	Tanzania	AF300902	-
licht64 Tang3	*lichtensteinii*	Tanzania	AF300903	-
licht65 Tang42	*lichtensteinii*	Tanzania	AF300904	-
licht66 Tang49	*lichtensteinii*	Tanzania	AF300905	-
licht67 Tang114	*lichtensteinii*	Tanzania	AF300906	-
cokei35 10.4.20.3	*cokii*	Kenya	AF300907	-
cokei46 Ug32	*cokii*	Uganda	AF300908	AF300939
lelwel2 Ug282	*lelwel*	Uganda	AF300909	AF300933
lelwel3 Ug321	*lelwel*	Uganda	AF300910	AF300934
lelwel4 Chad93	*lelwel*	Chad	AF300911	AF300935
lelwel32 58.135	*lelwel*	Sudan	AF300912	-
lelwel41 Cong27	*lelwel*	DRC	AF300913	-
lelwel42 Nig123	*lelwel*	Nigeria	AF300914	-
lelwel43 Ug263	*lelwel*	Uganda	AF300915	-
lelwel44 Ug445	*lelwel*	Uganda	AF300916	-
lelwel72 Chad94	*lelwel*	Chad	AF300917	-
lelwel73 Chad119	*lelwel*	Chad	AF300918	-
lelwel74 Chad153	*lelwel*	Chad	AF300919	-
neumanni	*neumanni*	unknown	AF300920	-
tora5 Abys I 128	*tora*	Sudan	AF300921	AF300940
tora33 58.136	*tora*	Sudan	AF300922	-
tora75 Abys I 126	*tora*	Sudan	AF300923	-
tora76 Abys I 129	*tora*	Sudan	AF300924	-
tora78 Abys I 130	*tora*	Sudan	AF300925	-
major6 Gui4B	*major*	Guinea	AF300926	AF300941
major7 Cam II 134	*major*	Cameroon	AF300927	AF300942
major57 Cam II 107	*major*	Cameroon	AF300928	-
major58 Cam II 137	*major*	Cameroon	AF300929	-
major79 Gui32	*major*	Guinea	AF300930	-
buselaphus	*buselaphus*	Northern Africa	AF300931	-
licht1 59520	*lichtensteinii*	-	-	AF016636
licht2 LHB2-97	*lichtensteinii*	Tanzania	-	AF034967
caama2 59517	*caama*	-	-	AF016640
caama1	*caama*	Zoo de Montpellier	-	AJ222681
caama22 SA22	*caama*	South Africa	-	AF300932

ID	Taxon	Country	GenBank accession no.	
Alcelaphus - outgroups				
Cgnou	*Connochaetes gnou*	-	-	AF016637
Ctaurinus	*Connochaetes taurinus*	-	-	AF034969
Dpygargus	*Damalisus pygargus*	-	-	AF036287
Hniger	*Hippotragus niger*	-	-	AF036285
Amelampus	*Aepyceros melampus*	-	-	AF022056
Nmoschatus1	*Neotragus moschatus*	-	-	AF022051
Nmoschatus2	*Neotragus moschatus*	-	-	FJ959387
Phacochoerus africanus[9]			**D-loop**	**D-loop**
GA1	*africanus*	Ghana	AY253760	AY253760
GA2	*africanus*	Ghana	AY253761	AY253761
GA3	*africanus*	Ghana	AY253762	AY253762
GA4	*africanus*	Ghana	AY253763	-
GA5	*africanus*	Ghana	AY253764	AY253764
GA6	*africanus*	Ghana	AY253765	AY253765
GA7	*africanus*	Ghana	AY253766	-
GA8	*africanus*	Ghana	AY253767	-
GA9	*africanus*	Ghana	AY253768	-
GA10	*africanus*	Ghana	AY253769	-
GA11	*africanus*	Ghana	AY253770	-
GA12	*africanus*	Ghana	AY253771	-
GA13	*africanus*	Ghana	AY253772	AY253772
GA14	*africanus*	Cameroon	AY253773	AY253773
GA15	*africanus*	Ghana	AY253774	AY253774
GA16	*africanus*	Ghana	AY253775	AY253775
NB1	*africanus*	Namibia	AY253776	-
GA18	*africanus*	Ghana	AY253777	-
GA19	*africanus*	Ghana	AY253778	-
GA20	*africanus*	Ghana	AY253779	-
NB1	*africanus*	Namibia	AY253780	-
NB2	*africanus*	Namibia	AY253781	AY253781
NB3	*africanus*	Namibia	AY253782	AY253782
NB4	*africanus*	Namibia	AY253783	-
NB5	*africanus*	Namibia	AY253784	-
ZBA1	*africanus*	Zambia	AY253785	-
ZBA2	*africanus*	Zambia	AY253786	AY253786
ZBA3	*africanus*	Zambia	AY253787	AY253787
ZBA4	*africanus*	Zambia	AY253788	-
ZBA5	*africanus*	Zambia	AY253789	-
ZB1	*africanus*	Zimbabwe	AY253790	-
ZB2	*africanus*	Zimbabwe	AY253791	AY253791
ZB3	*africanus*	Zimbabwe	AY253792	-
ZB4	*africanus*	Zimbabwe	AY253793	-

ID	Taxon	Country	GenBank accession no.	
Phacochoerus africanus[9]			**D-loop**	**D-loop**
ZB5	*africanus*	Zimbabwe	AY253794	AY253794
ZB6	*africanus*	Zimbabwe	AY253795	AY253795
ZB7	*africanus*	Zimbabwe	AY253796	-
ZB8	*africanus*	Zimbabwe	AY253797	AY253797
TZ1	*africanus*	Tanzania	AY253798	AY253798
TZ2	*africanus*	Tanzania	AY253799	AY253799
TZ3	*africanus*	Tanzania	AY253800	-
TZ4	*africanus*	Tanzania	AY253801	-
TZ5	*africanus*	Tanzania	AY253802	AY253802
TZ5	*africanus*	Tanzania	AY253803	-
TZ7	*africanus*	Tanzania	AY253804	-
TZ8	*africanus*	Tanzania	AY253805	AY253805
TZ9	*africanus*	Tanzania	AY253806	AY253806
TZ10	*africanus*	Tanzania	AY253807	-
TZ11	*africanus*	Tanzania	AY253808	-
TZ12	*africanus*	Tanzania	AY253809	-
TZ13	*africanus*	Tanzania	AY253810	AY253810
TZ14	*africanus*	Tanzania	AY253811	-
TZ14	*africanus*	Tanzania	AY253812	-
TZ16	*africanus*	Tanzania	AY253813	-
TZ17	*africanus*	Tanzania	AY253814	-
TZ18	*africanus*	Tanzania	AY253815	-
TZ19	*africanus*	Tanzania	AY253816	AY253816
KE1	*africanus*	Kenya	AY253817	AY253817
KE2	*africanus*	Kenya	AY253818	-
KE3	*africanus*	Kenya	AY253819	AY253819
UG1	*africanus*	Uganda	AY253820	-
UG2	*africanus*	Uganda	AY253821	-
UG3	*africanus*	Uganda	AY253822	-
UG4	*africanus*	Uganda	AY253823	AY253823
UG4	*africanus*	Uganda	AY253824	AY253824
UG6	*africanus*	Uganda	AY253825	-
UG7	*africanus*	Uganda	AY253826	AY253826
UG8	*africanus*	Uganda	AY253827	AY253827
UG9	*africanus*	Uganda	AY253828	-
UG10	*africanus*	Uganda	AY253829	-
Phacochoerus africanus - outgroups				
Paet1	*Phacochoerus aethiopicus*	Kenya	-	AJ314535
Bba2	*Babyrousa babyrussa*	-	-	AJ314546
Ssc1	*Sus scrofa*	-	-	AJ314542
Sve1	*Sus verrucosus*	-	-	AJ314538
Sbas1	*Sus barbatus*	-	-	AJ314540

1 Haus, T., Akom, E., Agwanda, B., Hofreiter, M., Roos, C. & Zinner, D. (2013) Mitochondrial diversity and distribution of African green monkeys (*Chlorocebus* Gray, 1870). *American Journal of Primatology*, **75**, 350-360.

2 Zinner, D., Groeneveld, L.F., Keller, C. & Roos, C. (2009) Mitochondrial phylogeography of baboons (*Papio* spp.): indication for introgressive hybridization? *BMC Evolutionary Biology*, **9**, e83.

3 Keller, C., Roos, C., Groeneveld, L.F., Fischer, J. & Zinner, D. (2010) Introgressive hybridization in southern African baboons shapes patterns of mtDNA variation. *American Journal of Physical Anthropology*, **142**, 125–136.

4 Arctander, P., Johansen, C. & Coutellec-Vreto, M.A. (1999) Phylogeography of three closely related African bovids (tribe Alcelaphini). *Molecular Biology and Evolution*, **16**, 1724–39.

5 Flagstad, O., Syvertsen, P.O., Stenseth, N.C. & Jakobsen, K.S. (2001) Environmental change and rates of evolution: the phylogeographic pattern within the hartebeest complex as related to climatic variation. *Proceedings of the Royal Society London B*, **268**, 667–677.

6 Hassanin, A. & Douzery, E.J. (1999) The tribal radiation of the family Bovidae (Artiodactyla) and the evolution of the mitochondrial cytochrome b gene. *Molecular Phylogenetics and Evolution*, **13**, 227–243.

7 Matthee, C.A. & Robinson, T. J. (1999) Cytochrome b phylogeny of the family bovidae: resolution within the Alcelaphini, Antilopini, Neotragini, and Tragelaphini. *Molecular Phylogenetics and Evolution*, **12**, 31–46.

8 Pitra, C., Kock, R.A., Hofmann, R. & Lieckfeldt, D. (1998) Molecular phylogeny of the critically endangered Hunter's antelope (*Beatragus hunteri* Sclater 1889). *Journal of Zoological Systematics and Evolutionary Research* **36**, 179–184.

9 Muwanika, V. B., Nyakaana, S., Siegismund, H.R. & Arctander, P. (2003) Phylogeography and population structure of the common warthog (*Phacochoerus africanus*) inferred from variation in mitochondrial DNA sequences and microsatellite loci. *Heredity*, **91**, 361–372.

CURRICULUM VITAE

Personal Information

Name	Tanja Haus
Date of birth	August 21, 1983
Place of birth	Bonn
Citizenship	German

Education

Since 2009	Ph.D. project, Cognitive Ethology and Primate Genetics Laboratories, German Primate Center (advisory board: Prof. Dr. Julia Fischer, Prof. Dr. Eckhard Heymann and Dr. Christian Roos)
Since 2009	Ph.D. program Biological Diversity and Ecology, University of Göttingen, Germany
2008	M.Sc. (Dipl.-Biol.), University of Bonn, Germany
	Thesis at the Zoologisches Forschungsmuseum Alexander Koenig (ZFMK), Bonn, Germany (supervised by Prof. Dr. Wolfgang Böhme): "Distribution and Population Densities of Primates in the Phong Nha-Ke Bang National Park, Central Vietnam, with focus on the Hatinh langur *Trachypithecus laotum hatinhensis* (Dao, 1970)", cooperation project of the Cologne Zoo and the Frankfurt Zoological Society
2002-2008	Studies in Biology, University of Bonn, Germany
2002	Abitur, Siebengebirgsgymnasium Bad Honnef, Germany

Grant

2009	Ph.D. grant Volkswagenstiftung, Initiative Evolutionary Biology (3.5 years)

Publications

Haus T, Akom E, Agwanda B, Hofreiter M, Roos C, & Zinner D. 2013. Mitochondrial diversity and distribution of African green monkeys (*Chlorocebus* Gray, 1870). *American Journal of Primatology 75*:350-360.

Nguyen Quang Truong, Böhme W, Nguyen Thien Tao, Le Khac Quyet, Pahl KR, **Haus T**, & Ziegler T. 2011. Review of the genus *Dopasia* Gray, 1853 (Squamata: Anguidae) in the Indochina subregion. *Zootaxa 2894*:58-68.

Haus T, Vogt M & Forster B. 2010. Primate censuses in difficult to access karst forests in the Phong Nha – Ke Bang National Park, Central Vietnam. In Nadler T, Rawson BM & Van Ngoc Thinh (eds): *Conservation of Primates in Indochina*. pp. 53-61.

Haus T, Vogt M & Forster B. 2009. Observations on the Hatinh langur *Trachypithecus hatinhensis* during point and line transect sampling in the Phong Nha – Ke Bang National Park, Central Vietnam. *Vietnamese Journal of Primatology 1*:17-27.

Haus T, Vogt M, Forster B, Vu Ngoc Thanh & Ziegler T. 2009. Distribution and population densities of diurnal primates in the karst forests of Phong Nha - Ke Bang National Park, Quang Binh Province, Central Vietnam. *International Journal of Primatology 30*:301-312.

EIDESSTATTLICHE ERKLÄRUNG

Hiermit erkläre ich, dass ich diese Dissertation selbstständig ohne Hilfe Dritter und ohne Benutzung anderer als der angegebenen Quellen und Hilfsmittel verfasst habe. Alle den benutzten Quellen wörtlich oder sinngemäß entnommenen Stellen sind als solche einzeln kenntlich gemacht.

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Des Weiteren erkläre ich, dass ich mich nicht anderweitig einer Doktorarbeit ohne Erfolg unterzogen habe und dass diese Arbeit in gleicher oder ähnlicher Form noch keiner anderen Prüfungsbehörde vorgelegen hat.

Göttingen, den	Tanja Haus	
Ort, Datum	Name	Unterschrift