ZENTRUM FÜR BIODIVERSITÄT UND NACHHALTIGE LANDNUTZUNG
SEKTION
BIODIVERSITÄT, ÖKOLOGIE UND NATURSCHUTZ
CENTRE OF BIODIVERSITY AND SUSTAINABLE LAND USE
SECTION: BIODIVERSITY, ECOLOGY AND NATURE CONSERVATION

Mitochondrial genomes and the complex evolutionary history of the cercopithecine tribe Papionini

Dissertation
zur Erlangung des Doktorgrades
der Mathematisch-Naturwissenschaftlichen Fakultäten
der Georg-August-Universität zu Göttingen

vorgelegt von

Dipl. Biol.
Rasmus Liedigk

aus
Westerstede

Göttingen, September 2014

Referent: PD Dr. Christian Roos
Korreferent: Prof. Dr. Eckhard Heymann
Tag der mündlichen Prüfung: 19.9.2014

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Chapter 1

General Introduction

1.1 An introduction to phylogenetics

First documented attempts of classifying the diversity of life go back to Aristotle around 2,400 years ago, who arranged organisms in a hierarchical way according to the complexity of their structure (Singer, 1931). Centuries later, in his *Systema Naturae* (1758) Carl Linnaeus was the first who categorised organisms by giving them genus and species names, and this binomial nomenclature is still applied in taxonomy and systematics nowadays (Beebee & Rowe 2008). A century after Linnaeus Alfred Russel Wallace (1858) and Charles Darwin (1859) developed the theory of evolution independently from each other and it was also Darwin (1859) and slightly later Ernst Haeckel (1866) who primarily depicted systematic relationships in tree-like illustrations (tree of life). Since that time, innumerable phylogenetic studies attempted to resolve how species are related to each other. One of the most commonly used approaches to classify organisms is the cladistic method. Basic ideas about cladistic analyses go back to the work of Willi Hennig (1950, 1966) after whom the aim of phylogenetics is to describe taxa that share common ancestry and therefore form monophyletic groups (clades). To detect such clades one has to distinguish ancestral (plesiomorphic) and derived (apomorphic) traits. Monophyletic clades are defined by shared derived characters (synapomorphies), while similarities that evolved independently from each other (homoplasy, convergence) can lead, if falsely interpreted, to incorrect relationships of respective taxa.

For a long time only morphological and anatomical traits were investigated to disentangle phylogenetic relationships. Later on, at the beginning of the twentieth century, different molecular techniques were applied, e.g., chromosome analysis or protein structure analysis (Pauling et al., 1951; Sutton, 1903). However, these techniques had their limits for taxonomic analyses (Avery et al., 1944; Dobzhansky, 1937; Watson & Crick 1953). In the 1970s and 1980s innovative methods like the Polymerase Chain Reaction (PCR) (Mullis et al., 1986) and Sanger sequencing

(Sanger et al., 1977) were introduced and revolutionised the field of molecular biology, because with these techniques it was possible to use DNA-sequence information for phylogenetic reconstructions. Additional advances and improvements of techniques such as high-throughput sequencing and DNA capturing now allow to efficiently generate DNA sequence data not only from high-quality and -quantity material (e.g., tissue, blood) but also from highly degraded DNA and small amounts of raw material (e.g., faeces, hair follicle, museum material).

Until recently DNA-based studies in primate phylogenetics were mainly based on single loci, including relative short DNA fragments of a few hundreds of base pairs (bp) such as non-recombining mitochondrial DNA sequences (e.g., Cytochrome b; Andrews et al., 1998; Haus et al., 2013; Roos et al., 2003; Thinh et al., 2010a; Yoder et al., 1996; Zhang & Ryder 1998; Ziegler et al., 2007). However, not all parts of the mammalian genome share the same mode of inheritance. While the Y chromosome is inherited only via the paternal lineage, the mitochondrial genome is passed on only maternally. Autosomes and the X chromosome are inherited from both parents. Recently, it turned out that phylogenies inferred from different loci often result in alternative tree topologies (e.g., Tosi et al., 2000; 2002; Roos et al., 2011). Hence, the topology of a tree based on one gene or locus has always to be regarded with caution. Gene trees are not necessarily species trees (Avise, 2004) and therefore multi marker approaches are carried out to compare the results and to infer the true species relationships (multi locus coalescence approach) (Degnan & Rosenberg 2009).

Phylogenetic analyses in primatology are generally of great interest, not only because primates are our closest relatives. Although primates are an extensively studied group, the field still offers astonishing new results concerning species delimitation and phylogenetic relationships. Even today there are still remote areas in Asia, Africa and South America which are understudied in terms of primate diversity and the respective phylogenetic relatedness of new or cryptic species. Molecular analyses are applied to solve phylogenetic relationships and to delimit species or taxonomic units. Such analyses can help to separate taxa that formerly were indistinguishable by morphological traits (cryptic species, e.g., mouse lemurs *Microcebus* spp., sportive lemurs *Lepilemur* spp., woolly lemurs *Avahi* spp., Andriaholinirina et al., 2006; Kappeler et al., 2005; Rabarivola et al. 2006; Tattersall, 2007; Zaramody et al. 2006). DNA sequence analyses led to the recent discovery of

new primate species (e.g., *Rhinopithecus strykeri, Trachypithecus cristatus selangorensis, Nomascus annamensis*) (Geissmann et al., 2011; Roos et al., 2008; Thinh et al., 2010b) and even a new genus (*Rungwecebus*, Davenport et al., 2006).

Phylogenetic studies however are not only important for taxonomic reasons. Almost all fields of biological research benefit from clarified and robust phylogenies. Comparative evolutionary studies, e.g., in behavioural ecology, are only meaningful if the underlying phylogeny is reliable (Pozzi et al., 2013). In biomedical research phylogenetic information is essential, e.g., to infer how and when a certain immunological disposition evolved. Knowledge about the phylogenetic relationships among non-human primates, especially in the Cercopithecinae, is of special interest since they represent important biomedical model organisms (Haus et al., in press; Smith et al., 2007).

To reconstruct phylogenetic relationships reliably, knowledge about species diversity and precise species delimitation is needed. If taxa or respective specimens are erroneously allocated to a wrong species, respective phylogenies are confounded and can lead to false conclusions. A clear taxonomy and phylogeny is also indispensable for comprehensive biodiversity assessments which provide the basis for the IUCN Red List of Threatened Species and for effective species conservation plans.

The general aim of my thesis is to investigate the phylogenetic relationships within the important cercopithecine tribe Papionini (mainly Asian macaques and African mangabeys and baboons).

1.2 Tribe Papionini – subfamily Cercopithecinae

The Old World monkeys or Cercopithecidae represent the only extant family in the catarrhine primate superfamily Cercopithecoidea. As inferred from fossil data and molecular studies the Cercopithecoidea diverged from the Hominoidea between 25-31 million years ago (Ma) (Chatterjee et al., 2009; Finstermeier et al., 2013; Perelman et al., 2011; Pozzi et al., 2011; Springer et al., 2012; Zalmout et al., 2010). The Cercopithecidae represent the most diverse family among all 16 primate families, including 23 genera and 159 species (Zinner et al., 2013). The Cercopithecidae consist of two subfamilies, the Colobinae (leaf monkeys) and the

Cercopithecinae (cheek pouch monkeys) (Groves, 2001; Zinner et al., 2013), which diverged from each other between 13 and 23 Ma (Chatterjee et al., 2009; Finstermeier et al., 2013; Perelman et al., 2011; Springer et al., 2012). Colobines comprise two tribes, the African Colobini and the Asian Presbytini, and the cercopithecines consist of the tribes Cercopithecini and Papionini (Groves, 2001; Zinner et al., 2013), which diverged during the Middle and Late Miocene, respectively (Perelman et al., 2011). The origin of the Papionini is most likely Africa and most extant species still inhabit most regions of sub-Saharan Africa. The Papionini comprise two subtribes, the Macacina (mainly Asian macaques) and the Papionina (African mangabeys and baboons).

1.2.1 Subtribe Papionina

The Papionina comprise six genera and 23 species (Zinner et al., 2013). The genus *Mandrillus* (mandrill and drill) consist of two species (*M. sphinx*, *M. leucophaeus*), the *Cercocebus* mangabeys comprise seven species (*C. galeritus, C. agilis, C. chrysogaster, C. sanjei, C. atys, C. lunulatus, C. torquatus*), the *Lophocebus* mangabeys six species (*L. albigena*, *L. osmani*, *L. johnstoni*, *L. ugandae*, *L. aterrimus*, *L. opdenboschi*). The genera *Rungwecebus* and *Theropithecus* are monotypic and include the kipunji or highland mangabey (*R. kipunji*) and the gelada (*T. gelada*) respectively. The genus *Papio* currently comprises six species (*P. papio*, *P. hamadryas*, *P. ursinus*, *P. anubis*, *P. kindae*, *P. cynocephalus*).

The Papionina are geographically widespread and ecologically diverse (Harris, 2000). Among them one finds predominantly arboreal (*Lophocebus, Rungwecebus*) and terrestrial taxa (*Cercocebus*, *Mandrillus*, *Papio, Theropithecus*) (Geissmann, 2003; Zinner et al., 2013). Papionin taxa inhabit a variety of different habitat types which range from rainforest (*Mandrillus, Cercocebus*, *Lophocebus, Rungwecebus*) to savannah and semi-desert (*Papio*) and to mountainous regions (*Theropithecus, Papio*) (Zinner at al., 2013b). Within the Papionina, *Papio* is the only genus which extended its range beyond the borders of the African continent to south-western Arabia.

The genus *Papio* (Erxleben, 1777) is mainly an African group inhabiting large regions of the sub-Saharan part of the continent. As indicated by fossil records (Jablonski & Frost, 2010; Williams et al., 2012) and genetic studies (Keller et al.,

2010; Newman et al., 2004; Sithaldeen et al., 2009; Zinner et al., 2009; Zinner et al., 2013), the genus originated in southern Africa at around 2 Ma. The classification of the various *Papio* taxa is disputed. Groves (2001) and Grubb et al. (2003) proposed five species (*P. hamadryas*, *P. papio*, *P. anubis*, *P. cynocephalus*, *P. ursinus*) while earlier studies combined all taxa in just a single species (*P. hamadryas* or *P. cynocephalus*). The Kinda baboon was formerly considered a subspecies of the yellow baboon (*P. cynocephalus kindae*) (Groves, 2001; Kingdon 1997), but due to morphological (Frost et al., 2003) and genetic distinctiveness (Burrell, 2009; Zinner et al., 2009a) it was recently proposed as full species (Jolly et al., 2011; Zinner et al., 2013a). However, phylogenetic relationships among these six baboon taxa remain unclear. Phylogenetic studies based on molecular data yielded several cases of paraphyly and even polyphyly among *Papio* taxa. Zinner et al. (2009a) obtained seven major haplogroups, but phylogenetic relationships among them were not fully resolved. Further the obtained clades rather reflect the geographic distribution of respective taxa and do not correspond to the taxonomic classification (Keller et al., 2009; Zinner et al., 2009a; Zinner et al., 2009b).

Due to its vast distribution throughout savannah habitats and an evolution temporally in parallel to humans, baboons have been regarded as model taxon to understand early human dispersal scenarios (Garrigan & Kingan, 2007; Jolly, 2001; Kopp et al. in press; Newman et al., 2004) and it is therefore of special interest to elucidate phylogenetic relationships within this genus.

1.2.2 Subtribe Macacina, genus *Macaca*

The genus *Macaca* (Lacépède, 1799) is, with the exception of *Papio*, the only papionin genus with extant members outside Africa (Evans et al., 1999; Groves, 2001; Zinner et al., 2013). Fossil data suggest that macaques arose in Northeast Africa approximately 7 Ma and began their evolutionary diversification about 5.5 Ma, spreading north and eastward into Eurasia (Delson, 1975, 1980, 1996). During this range expansion, the genus diversified into distinct species groups that are variously defined along biogeographic, morphological and molecular lines (Delson, 1980; Riley, 2010; Tosi et al., 2003). Hence, macaques are one of the most successful extant primate radiations in terms of range expansion and diversity. The genus is highly speciose, is found in over 20 Asian countries and parts of Northern Africa and

it covers an area of more than 5 million km^2 (Tosi et al., 2003). The range of colonised habitats, from continents to islands, is unique among non-human primates (Abegg & Thierry, 2002) and makes the genus *Macaca* an excellent example of adaptive radiation among primates (Riley, 2010).

The genus *Macaca* comprises 20 - 24 species depending on the classification of different authors (Groves, 2001; Tosi et al., 2003; Ziegler et al., 2007; Zinner et al., 2013) that are grouped into several species groups. Based on genital morphology of male macaques Fooden (1976) proposed four extant species groups that are: (1) the *sylvanus-silenus* group (*M. sylvanus*, *M. silenus*, *M. nemestrina*, Sulawesi macaques), (2) the *fascicularis* group (*M. fascicularis*, *M. mulatta, M. fuscata*, *M. cyclopis*), (3) the *sinica* group (*M. sinica*, *M. radiata*, *M. assamensis*, *M. thibetana*) and (4) the monotypic *M. arctoides* group. By analysing cranial morphology Delson (1980) modified this classification by placing *M. arctoides* as a member of the *sinica* group and removing *M. sylvanus* from the *silenus* group to form a sister taxon to all other macaques. Taking both morphological and genetic data into account, Groves (2001) divided the genus into six species groups, (1) the monotypic *M. sylvanus* group, (2) the *M. nemestrina* group (*M. nemestrina*, *M. leonina*, *M. silenus*, *M. pagensis*), (3) the Sulawesi group, (4) the *M. fascicularis* group (*M. fascicularis*, *M. arctoides*), (5) the *M. mulatta* group (*M. mulatta*, *M. cyclopis*, *M. fuscata*) and (6) the *M. sinica* group (*M. sinica*, *M. radiata*, *M. assamensis*, *M. thibetana*). In contrast to Groves (2001), Zinner et al. (2013) separated *M. fascicularis* from *M. arctoides* and allocated both in monotypic groups, thus recognising a total of seven species groups.

Fooden (1976, 1980) proposed that macaques dispersed in three successive waves what he inferred from their present-day distribution. As the *sylvanus-silenus* (+ Sulawesi) lineage inhabits the most fragmented distribution, it was assumed to be the first that dispersed. The *sinica-arctoides* lineage with its moderately fragmented distribution was proposed to have dispersed secondly and the *fascicularis* (+ *mulatta*) lineage third as it has the most broadly continuous distribution (Fooden, 1976, 1980). Despite the general consensus about the above-mentioned lineages and their dispersal, the phylogenetic relationships among species and species groups have not been conclusive. A number of issues concerning relatedness and dispersal routes within Southeast Asia and the Sunda Shelf remain to be clarified.

Of special interest among macaques is the *M. fascicularis* group which is one of the youngest macaque lineages. The monotypic group (sensu Zinner et al., 2013)

has beside rhesus macaques the largest distribution and it is found throughout southern Southeast Asia, the Sunda Shelf and beyond as far as the Philippines and the island of Timor. In the northern part of its range *M. fascicularis* is introgressed by the parapatric *M. mulatta* (Bonhomme et al., 2009; Tosi et al., 2000; Tosi et al., 2002; Tosi & Coke 2007; Zinner et al., 2013). About 30% of the mainland *M. fascicularis* genome is of *M. mulatta* origin (Yan et al., 2011). Reconstruction of phylogenetic relationships based on molecular data showed a clear division into a continental and an insular *fascicularis* clade (Tosi et al., 2003), but when exactly both, the continental and insular populations diverged, is unclear. Inconsistencies of divergence times as inferred from mitochondrial and Y-chromosomal data, and the fact that both continental and insular genotypes are present on Sumatra support the hypothesis that both populations were in contact for several thousand years (Tosi et al., 2003; Tosi & Coke 2007). The origin of the *fascicularis* group is unclear, but Delson (1980) suggested that macaques entered Sundaland during periods of low sea level. Pleistocene fossils from Java indicate that proto-*fascicularis* probably became isolated on Java and later extended its range again to the North (Delson, 1980). Further dispersal routes of *M. fascicularis* in Sundaland are not known and it is unclear whether the taxon extended its range to Timor by itself or whether humans introduced it there.

1.3 Papionin fossils in Europe and Asia

Having dispersed from Africa during the Late Miocene, several taxa of the cercopithecines existed in Europe during the Pliocene and the Early Pleistocene (Alba et al., 2014; Delson, 1974; Köhler et al., 2000). Fossil remains from outside of Africa that are assigned to recent papionin genera comprise solely macaques and members of the genus *Theropithecus*, whereas fossil remains of other extant papionin genera are only known in Africa. *Cercocebus* fossils have been recovered in South Africa, Kenya and Tanzania from Late Pliocene localities. Plio-Pleistocene remains of *Lophocebus* were also found in Kenya. Plio- and Pleistocene fossils, that were assigned to *Papio* are known from South and East Africa (Hartwig, 2002). In the following paragraph I give an overview about papionin fossils that were found outside of Africa.

1.3.1 Fossils of *Macaca*

Fossil macaque remains have been found in southern and central Europe as well as in Asia. The European remains, mainly teeth and partial jaws, were designated to *Macaca sylvanus prisca* (Gervais, 1859; Delson, 1980) and were found in some cases in assemblages together with early colobines (*Mesopithecus*) (Alba et al., 2014; Delson, 1980). To date, probably the oldest macaque fossils outside Africa were discovered in Late Miocene localities in eastern Spain (Köhler et al., 2000) and northern Italy (Alba et al., 2014). Although not all European fossils have been assigned to the species level, most of them are considered to belong to the *M. sylvanus* lineage (Alba et al., 2014). Dental morphology of the European macaques do not differ much from *M. lybica* (Stromer, 1920) which was discovered in a Late Miocene excavation from northern Egypt, indicating affiliation to the same lineage (*M. sylvanus*). Taking together the information about earliest macaque fossils in Europe, it is assumed that a dispersal of the genus, and cercopithecines in general, out of Africa took place at the Miocene – Pliocene boundary (Alba et al., 2014). Recently, a ~6.5 - 8 million-years-old guenon fossil (Cercopithecini) was discovered in Arabia (Gilbert et al., 2014). Although guenons belong to the sister tribe of the Papionini, this finding indicates that dispersal of cercopithecines out of Africa might have occurred earlier than previously thought.

Compared to the fossil record of Europe, macaque associated remains from Asia are rather scarce (Delson, 1980). The first cercopithecid fossils in Asia were, as in Europe, colobines and the oldest fossil associated with *Macaca* were found in China (~4 Ma) (Alba et al., 2014) and in northern India (~3 Ma) (Delson, 1980). All other macaque fossils from Asia are from the Pleistocene. Dental remains from the Early Pleistocene that were found in China were named *M. anderssoni* and *M. robusta* (Schlosser, 1924; Young, 1934), whereas the former is similar to the modern *M. thibetana* and *M. arctoides* (Delson, 1980). The youngest Chinese macaque fossil is from the Late Pleistocene and can be referred to *M. mulatta*. A Middle Pleistocene fossil that is also associated with *M. thibetana* and *M. arctoides* was found in northern Vietnam (Jouffroy, 1959). Fossil macaques from Java (Middle Pleistocene) are not precisely identified but may be associated with *M. fascicularis* or *M.*

nemestrina (Delson, 1980). Further remains have been recovered in South Korea where macaques are absent nowadays.

1.3.2 Fossils of *Theropithecus*

The fossil record of *Theropithecus* reveals that the genus has been quite abundant in the Plio-Pleistocene of Africa (Delson, 1993; Delson et al., 1993; Roberts et al., 2014; Rook et al., 2004), whereas it is nowadays restricted to the highlands of Ethiopia (Jablonski & Frost, 2010). *Theropithecus* fossils, which have been recovered from north-eastern Ethiopia, were allocated to *T. oswaldi cf. darti*. These remains have been dated to between 3.6 and 3.8 Ma and were found in an assemblage with other cercopithecines and colobines (Frost et al., 2014). During the Early Pleistocene, much later than macaques, *Theropithecus* extended its range from Africa, via the Near East (~1.4 Ma, Belmaker, 2002; Rook et al., 2013) to India (~1.0 Ma), southern Italy and Spain (~1.0 Ma) (Delson, 1993; Delson et al., 1993; Gibert et al., 1995; Gupta & Sahni, 1981; Pickford, 1993; Roberts et al., 2014; Rook et al., 2004, 2013). *Theropithecus* remains from Southeast Asia are not reported.

1.4 The mitochondrial genome and its application in phylogenetics

Most eukaryotic cells contain mitochondria in the cytoplasm. These organelles, which serve as chemical power supplies for the cell, comprise their own DNA. The mitochondrial DNA (mtDNA) evolves independently from the nuclear DNA (nDNA) and is inherited only via the maternal lineage since sperm cells do not contribute any mitochondria to the zygote. The circular, double-stranded mtDNA molecule in vertebrates is around 16.5 kilobases (kb) in length and consists of 37 genes coding for two rRNA, 22 tRNAs and 13 proteins and includes also the non-coding control region (Beebee & Rowe, 2008; Wolstenholme, 1992).

Analysing mtDNA in the context of phylogenetic studies provides several advantages. Each mitochondrion contains two to ten copies of mtDNA and each cell contains up to several thousand mitochondria, hence the number of copies of mtDNA per cell is much higher than that of nDNA (Wiesner et al., 1992). This is of particular importance when working with degraded DNA as found e.g. in faeces or ancient

material like museum specimens or sub-fossil material. Another advantage is that the mutation rate in vertebrate mtDNA is five to ten times higher than in nDNA, which makes it a useful molecular marker to study closely related taxa and inter-specific relationships (Beebee & Rowe, 2008).

As mentioned earlier, mtDNA is only inherited maternally. Since most primate taxa live in female philopatric societies, in which males disperse and females stay in their natal groups, mtDNA can reveal insights into genetic differences among regional populations (Smith et al., 2007). Furthermore, the instance of maternal inheritance can also help to trace back the geographical origin of respective taxa (Avise, 2004), at least on an intra-specific level where splits between taxa are relatively young.

Generally, the mutation rate in mtDNA is higher than in nDNA, but the nucleotide substitution rate also varies within the mitochondrial genome. Therefore different parts of the mitochondrial genome have been used to reconstruct phylogenetic relationships among primate taxa. Several studies used the less variable cytochrome b gene (Andrews et al., 1998; Haus et al., 2013; Roos et al., 2003; Thinh et al., 2010b; Yoder et al., 1996; Zhang & Ryder 1998; Ziegler et al., 2007) to estimate affiliations among closely related primate taxa. In other studies that focused more on genetic diversity within and among populations, the highly variable control region was investigated (e.g., Ebenau et al., 2011; Yang et al., 2012).

While working with mtDNA care has to be taken not to amplify nuclear pseudogenes, the so-called numts (nuclear mitochondrial sequences). Numts are copies of mtDNA that are integrated into the nDNA where they evolve independently from the true mtDNA. The amplification of these numts can result in confounded phylogenies (Thalmann et al., 2004) and therefore have to be avoided. The chance to amplify numts is relatively low when using DNA extracted from faecal samples or museum specimens since nDNA is normally highly degraded in such material.

1.5 Divergence time estimation using molecular data

DNA sequence data can be used to estimate divergence times of certain lineages. The underlying concept of a molecular clock (Zuckerkandl & Pauling, 1965) means that the nucleotide substitution rate within a certain DNA region is constant among all

lineages. The degree of divergence between two lineages is assumed to be directly proportional to the time since divergence. In the meantime it became clear that evolutionary rates vary among different taxa depending on e.g. population size, body size, metabolic rate and generation time (Martin & Palumbi, 1993), especially when deep splits are considered. Therefore a relaxed molecular clock approach, which addresses these differences, is in most cases applied instead of a strict molecular clock model. Regardless of which clock model is favoured, all molecular phylogenies, which incorporate divergence date estimation, need to be calibrated. Normally this is done with fossils. Therefore the respective fossil record has to be browsed for specimens that reliably document the occurrence of a particular taxon in a certain time range. These data can be used to fix respective nodes in a phylogeny of interest, what is essential for reliable time estimations.

1.6 Aims of the study

On the basis of complete mitochondrial genome sequences I aim to reconstruct phylogenetic relationships on different taxonomic levels within the Papionini. By investigating inter- and intra-generic as well as intra-specific relationships a broad time range of the evolutionary history of the Papionini is taken into account.

1. In the first study (chapter 2) I combine representatives from all genera and species groups of the Papionini (with the exception of *Rungwecebus*) in one comprehensive phylogeny and estimate divergence times. In this context relationships within the *Mandrillus* – *Cercocebus* and within the *Theropithecus* – *Lophocebus* – *Papio* clade, as well as relationships among macaque species groups are of special interest since previous studies depicted contradicting relationships. For this study, I did all laboratory work, analysed data together with Markus Brameier (MB), Christian Roos (CR) and Dietmar Zinner (DZ), and wrote the paper together with CR and DZ.

2. In the second study (chapter 3) we investigate affiliations among different populations and species of the African genus *Papio* to test whether complete mtDNA genomes reveal a better resolution of phylogenetic relationships than previous analyses using only short mtDNA sequences. For this study, I did laboratory work

together with Jenny Wertheimer, and analysed data and wrote the paper together with Linn F. Groeneveld, DZ and CR.

3. In the third study (chapter 4) we analyse the intra-specific relationships within *Macaca f. fascicularis* by reconstructing a mitogenomic phylogeny using samples from throughout the subspecies' range. The split between mainland and Sundaland populations as well as possible dispersal routes are of special interest. The study incorporates sequence data inferred from traditional Sanger sequencing as well as from a DNA capture method followed by high-throughput sequencing. Hence, we are able to compare the accuracy of both methods. For this study, I did laboratory work together with Jakob Kolleck (JK) and Kai Böker (KB), analysed data together with JK, KB, MB, DZ and CR, and wrote the paper together with DZ and CR.

Chapter 2

Mitogenomics of the Old World monkey tribe Papionini

Rasmus Liedigk[1*], Christian Roos[1,2], Markus Brameier[1], Dietmar Zinner[3]

[1] Primate Genetics Laboratory, German Primate Center, Leibniz Institute for Primate Research, Kellnerweg 4, 37077 Göttingen, Germany

[2] Gene Bank of Primates, German Primate Center, Leibniz Institute for Primate Research, Kellnerweg 4, 37077 Göttingen, Germany

[3] Cognitive Ethology Laboratory, German Primate Center, Leibniz Institute for Primate Research, Kellnerweg 4, 37077 Göttingen, Germany

[*] Correspondance: rliedigk@gmx.de

Accepted for publication in BMC Evolutionary Biology, 25 July 2014.

Abstract

Background

The evolutionary history of the Old World monkey tribe Papionini comprising the genera *Macaca*, *Mandrillus*, *Cercocebus*, *Lophocebus*, *Theropithecus*, *Rungwecebus* and *Papio* is still matter of debate. Although the African Papionini (subtribe Papionina) are generally considered to be the sister lineage to the Asian Papionini (subtribe Macacina), previous studies based on morphological data, nuclear or mitochondrial sequences have shown contradictory phylogenetic relationships among and within both subtribes. To further elucidate the phylogenetic relationships among papionins and to estimate divergence ages we generated mitochondrial genome data and combined them with previously published sequences.

Results

Our mitochondrial gene tree comprises 33 papionins representing all genera of the tribe except *Rungwecebus*. In contrast to most previous studies, the obtained phylogeny suggests a division of the Papionini into three main mitochondrial clades with similar ages: 1) *Papio*, *Theropithecus*, *Lophocebus*; 2) *Mandrillus*, *Cercocebus*; and 3) *Macaca*; the *Mandrillus* + *Cercocebus* clade appears to be more closely related to *Macaca* than to the other African Papionini. Further, we find paraphyletic relationships within the *Mandrillus* + *Cercocebus* clade as well as in *Papio*. Relationships among *Theropithecus*, *Lophocebus* and *Papio* remain unresolved. Divergence ages reveal initial splits within the three mitochondrial clades around the Miocene/Pliocene boundary and differentiation of *Macaca* species groups occurred on a similar time scale as those between genera of the subtribe Papionina.

Conclusion

Due to the largely well-resolved mitochondrial phylogeny, our study provides new insights into the evolutionary history of the Papionini. Results show some contradictory relationships in comparison to previous analyses, notably the paraphyly within the *Cercocebus* + *Mandrillus* clade and three instead of only two major mitochondrial clades. Divergence ages among species groups of macaques are

similar to those among African Papionini genera, suggesting that diversification of the mitochondrial genome is of a similar magnitude in both subtribes. However, since our mitochondrial tree represents just a single gene tree that most likely does not reflect the true species tree, extensive nuclear sequence data is required to illuminate the true species phylogeny of papionins and to trace possible ancient hybridization events among lineages.

Keywords

Phylogeny, Divergence ages, mtDNA, Primates, Macaques, Baboons

Background

It is well recognized that mitochondrial (mtDNA) phylogenies are not necessarily congruent with the phylogeny of the respective taxa or phylogenies based on a set of nuclear genes (e.g. [1-3]). Reasons for the incongruence are manifold, e.g., different inheritance pathways, divergent selection pressures, and most prominent, incomplete lineage sorting and horizontal gene flow (e.g. [4,5]). On the other hand, if mtDNA and nuclear (nDNA) phylogenies are congruent this could be a strong indication that the single underlying gene tree is congruent with the species tree. Furthermore, in many species analyses of mtDNA relationships provide a better spatial resolution, thus contributing to phylogeographical inferences [3,6]. Therefore, analyses of both, mtDNA and nDNA, are necessary for a comprehensive understanding of the evolutionary history of taxa and for a robust reconstruction of complex phylogenies.

Among primates, incongruences are reported for several taxa within the Old World monkey tribe Papionini (e.g. [7-14]). The Papionini tribe diverged from its sister lineage, the Cercopithecini, around 11.5 million years ago (Ma) [15] and is comprised of the subtribe Papionina, with the genera *Papio*, *Mandrillus*, *Theropithecus*, *Cercocebus*, *Rungwecebus* and *Lophocebus*, and the subtribe Macacina, with the genus *Macaca* [16]. While all available nDNA data and respective gene trees are congruent and strongly support this division [15,17,18], recent studies applying

mtDNA genome data suggest the *Mandrillus* + *Cercocebus* clade to be closer related to *Macaca* [19,20], thus indicating paraphyly of Papionina in the mtDNA gene tree.

The African origin of the tribe is broadly accepted [16,21-25] and the fossil record indicates a Late Miocene dispersal out of Africa into Eurasia for some lineages. Remains of macaques have been found in southern, western and central Europe [26,27], whereas fossil macaques from Asia are documented but rather scarce [26]. Fossils of *Theropithecus* have been recovered from the Iberian Peninsula as well as from India [28-34]. The six genera of Papionina are found today exclusively on the African continent, with the exception of the hamadryas baboon, which occurs in both northeastern Africa and the southwestern Arabian Peninsula [16,25]. In contrast, members of the subtribe Macacina are distributed over large regions of South, Southeast and East Asia with the exception of Barbary macaques, which are found in Northwest Africa. Based on morphological characters, the subtribe Papionina is divided into six relatively heterogeneous genera, while the Asian lineage consists of only one highly speciose genus (*Macaca*), which is divided into several species groups [16,23,26,35].

The tribe comprises 45 species [36], exhibiting a great variety of morphologies from more slender representatives like the crested mangabeys to more robust forms like baboons, mandrills and drills. The genus *Macaca* is divided into species groups, but the number and the composition of these species groups have been a matter of debate for decades [23,26,35]. Based on the morphology of male genitals Fooden [35] proposed four species groups comprising a *M. silenus-M. sylvanus*, a *M. fascicularis*, a *M. arctoides* and a *M. sinica* group, with a total of 19 species. Delson [26] also proposed four species groups but moved *M. arctoides* into the *M. sinica* group and separated *M. sylvanus* from the *M. silenus* lineage into its own group. Combining morphological and genetic data, Groves [23] proposed a classification into six species groups with a total of 20 species: (1) the monotypic *M. sylvanus* group, (2) the *M. nemestrina* group, (3) the Sulawesi group, (4) the *M. fascicularis* group, (5) the *M. mulatta* group and (6) the *M. sinica* group. In the most recent classification the genus *Macaca* consists of 22 species, which are divided into seven species groups [16], among them three monotypic species groups: (1) *M. sylvanus* group, (2) *M. arctoides* group and (3) *M. fascicularis* group, and four polytypic groups: (4) Sulawesi group, (5) *M. mulatta* group, (6) *M. sinica* group and (7) *M.*

silenus group. Although the monophyly of the macaques was confirmed in several studies [23,26,35,37,38], relationships among and within the species groups are still disputed [37-40].

Similarly, within the African Papionina, relationships among genera and species are only partly resolved [41]. Findings based on morphological traits were often discordant with results from molecular studies. While early morphological analyses supported the monophyly of the mangabeys [42,43], more recent morphological [44-46] and molecular studies [17,47,48] suggested diphyly of mangabeys, with *Lophocebus* clustering with *Papio* and *Theropithecus*, while *Cercocebus* forms a clade with *Mandrillus*. The kipunji (*Rungwecebus kipunji*), earlier described as a member of *Lophocebus* [49], was recently placed in its own genus [50]. Subsequent genetic studies confirmed the diphyly of *Lophocebus* and *Cercocebus*, and in addition showed a close relationship of *Rungwecebus* to *Papio* [10,50,51]. Concerning *Papio*, genetic analyses revealed seven well-supported mtDNA haplogroups, but these were not congruent with the six recognized species of the genus [11,42,52-54]. Likewise, for the *Mandrillus* + *Cercocebus* clade a mtDNA study indicated paraphyly of *Cercocebus* with at least one species (*C. torquatus*) being more closely related to *Mandrillus* than to its congenerics [12], while nuclear gene trees suggest reciprocal monophyly of both genera [14,15]. Previous morphological studies noted some similarities between *Mandrillus*, *Cercocebus* and *Macaca*. Fleagle and McGraw [45,55] studied postcranial features of *Mandrillus*, *Cercocebus*, *Lophocebus* and *Papio* and compared them with respective data of one macaque species (*M. nemestrina*). Most characters of *Mandrillus* and *Cercocebus* did not differ from those of *M. nemestrina*, and were therefore interpreted to be primitive among papionins, whereas just one of the investigated traits in *M. nemestrina* did not differ from that of *Lophocebus*, *Papio* and *Theropithecus* [45,55]. Furthermore, although it is widely accepted that *Lophocebus* and *Theropithecus* cluster together with a clade consisting of *Papio* and *Rungwecebus*, the branching pattern among these lineages is unresolved [14,19,20,56].

It has recently been shown that the use of complete mtDNA genome sequences provide better statistical support in phylogenetic reconstructions when compared to analyses based on single genes or partial genomes (e.g. [57-60]). In our study we generated new mtDNA genome data of *Macaca* species and combined it with

respective data of other Papionini from GenBank to reconstruct a robust mtDNA gene tree of papionin primates and to estimate respective divergence ages. We were particularly interested to obtain further information concerning the branching pattern among papionin genera and among all seven species groups of the genus *Macaca* and to provide comprehensive data for further comparative molecular studies.

Results

We sequenced complete mtDNA genomes from eight macaques representing all seven macaque species groups: *M. sylvanus* – *M. sylvanus* group, *M. silenus* – *M. silenus* group, *M. tonkeana* – Sulawesi group, *M. thibetana* – *M. sinica* group, *M. mulatta*/China and *M. mulatta*/India – *M. mulatta* group, *M. fascicularis*/Vietnam – *M. fascicularis* group, and *M. arctoides* – *M. arctoides* group. A BLAST-search in GenBank showed that our newly generated sequences matched almost perfectly with available orthologs. The full-length genome sequences consisted of 13 protein-coding genes, 2 rRNA genes, 22 tRNA genes and the control region. The initial alignment comprised 38 sequences and had a length of 16,966 bp. After indels and poorly aligned positions were removed the alignment comprised 15,685 bp including 6,986 informative sites. The alignment is available for download (Additional file 1 [61]).

The phylogenies as obtained from maximum-likelihood (ML) and Bayesian analyses are mainly identical and most branching patterns are strongly supported (Figure 1). Likewise, the Densitree [62] depicting the posterior distribution of the 25,000 trees as inferred from the Bayesian divergence age analysis in BEAST suggests the most frequent tree topology to be identical to that obtained from ML and Bayesian analyses (Figure 2). According to divergence age estimations using auto-correlated and uncorrelated clock models, the Old World monkeys (Cercopithecoidea) diverged from the Hominoidea between 24 and 27 Ma (for 95% credibility intervals see Additional file 2: Table S1). In the Early Miocene, the two subfamilies of the Cercopithecidae, Colobinae and Cercopithecinae, separated, and the latter further split into Cercopithecini and Papionini between 11 and 16 Ma. Our analysis revealed three major clades within the Papionini which diverged 9–13 Ma. Interestingly, the *Mandrillus* + *Cercocebus* clade forms a sister lineage to *Macaca* (ML bootstrap value [BP]: 100%; Bayesian posterior probability [PP]: 1.0) and does not cluster with the

second major African papionin clade comprising *Papio, Lophocebus* and *Theropithecus* (BP: 100%; PP: 1.0). Since *Mandrillus* and *Cercocebus* show a shift in A/C content similar to macaques (Additional file 3: Figure S1), which could lead to an artificial clustering [63], we repeated our analysis with a modified dataset (dataset 2) that corrects for this shift. Accordingly in this second alignment we masked positions that contain both an Adenin and Cytosin with an "M". The resulting overall branching pattern and specifically the phylogenetic position of the *Mandrillus* + *Cercocebus* clade among papionins were identical to those obtained from the original dataset (Additional file 4: Figure S2). To further test for alternative positions of the *Mandrillus* + *Cercocebus* clade among papionins, we performed alternative tree topology tests, which revealed that all alternative options are statistically rejected (Figure 3).

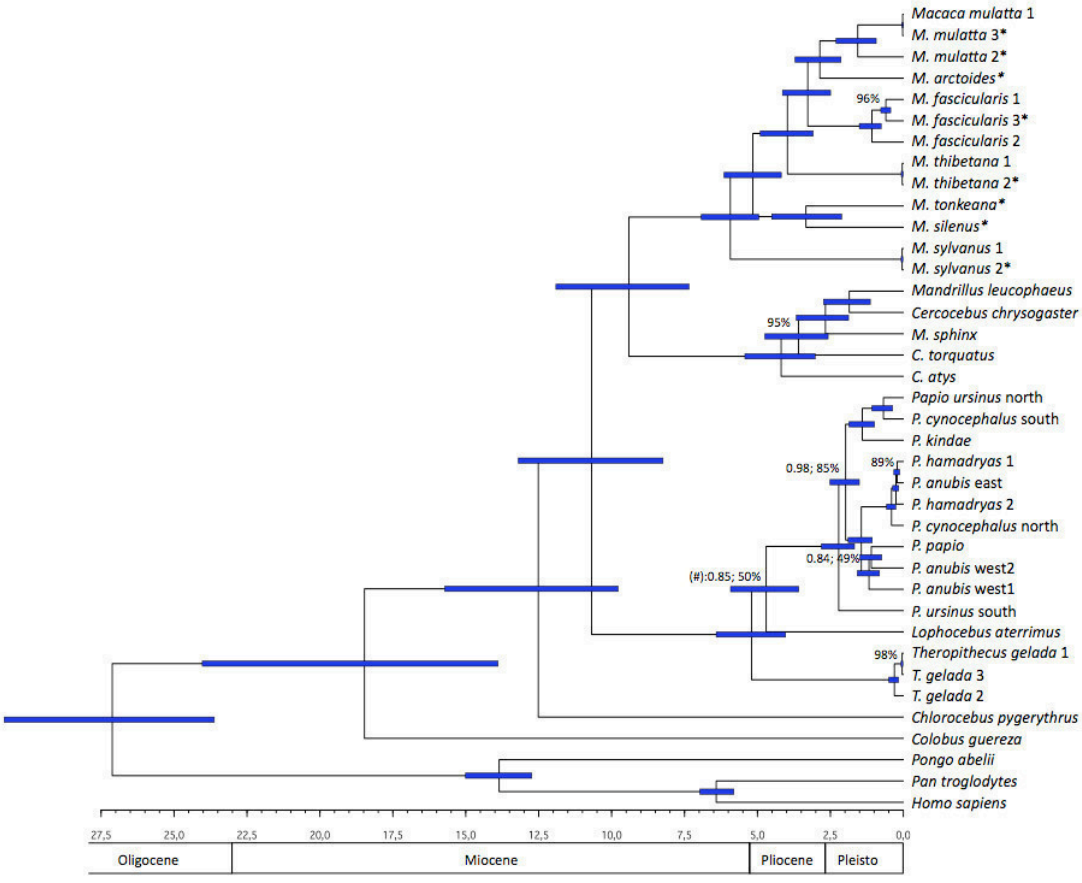

Figure 1 Ultrametric tree of the Papionini and outgroup taxa as inferred from mtDNA dataset 1. Tree topologies as inferred from Bayesian (MrBayes) as well as from ML (RAxML) estimation were identical with one exception: At one node (labelled with #) the ML tree indicates Lophocebus as sister lineage to the Papio + Theropithecus clade (not depicted). All unlabelled branches show ML BP of 100% and Bayesian PP of 1.0. Values below are indicated at respective nodes. Blue bars indicate 95% credibility intervals of divergence ages. Time scale shows million years before present. For information about taxa and samples see Additional file 7: Table S2. * = sequences were newly generated in this study.

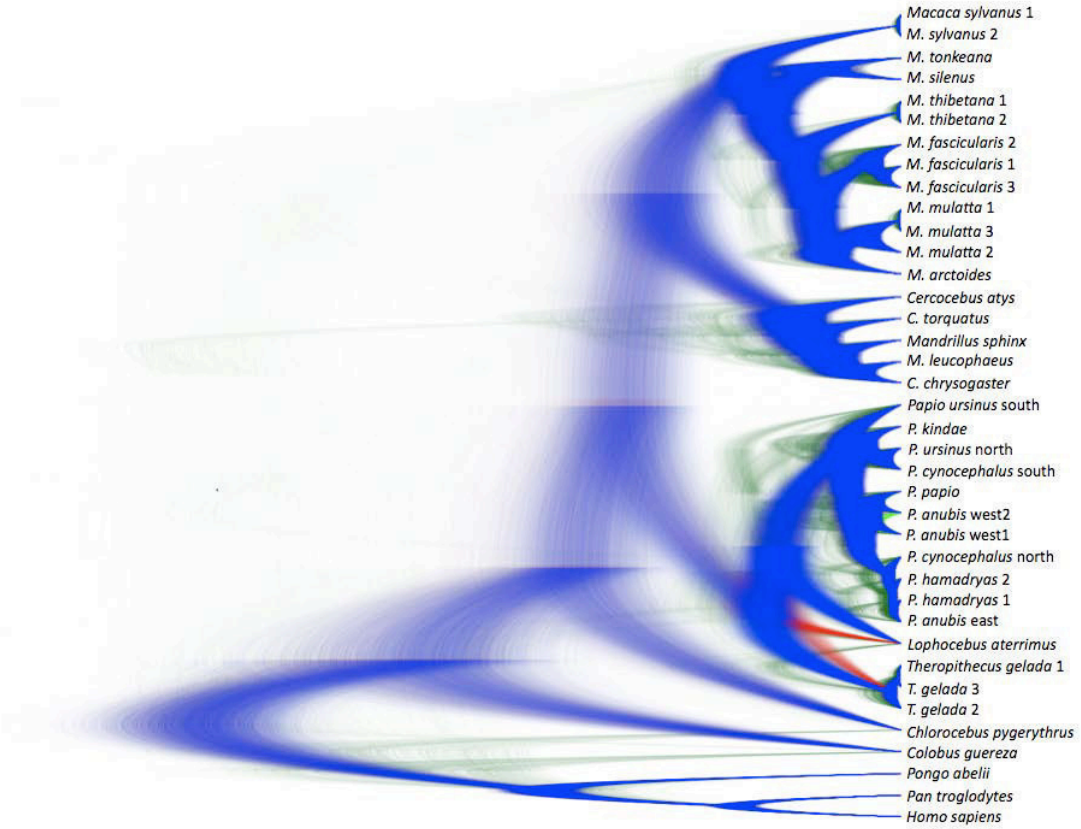

Figure 2 Densitree showing the posterior probability of 25,000 trees taken from the Bayesian divergence age analysis in BEAST. Blue represents the most frequent tree topology, red represents the second and green the third most frequent topology.

Within the *Mandrillus* + *Cercocebus* clade, members of both genera do not form reciprocally monophyletic clades. In dataset 1 *C. atys* is the first lineage to split off (4.2-4.9 Ma) followed by *C. torquatus* (3.6-4.3 Ma), while *M. sphinx* represents a sister lineage to *C. chrysogaster* and *M. leucophaeus* (BP: 100%; PP: 1.0) which separated from them 2.7-3.4 Ma. The latter two diverged 1.9-2.6 Ma. The Bayesian analysis of dataset 2 shows the same topology, but partly with low support (PP: 0.56) while the ML analysis of dataset 2 suggests a possible clade consisting of *C. atys* and *C. torquatus* but only weakly supported (BP: 49%) (Additional file 4: Figure S2).

Within the second African papionin clade, the branching pattern among the three genera *Papio*, *Theropithecus* and *Lophocebus* is not well resolved. While in the Bayesian analysis of the original dataset, *Theropithecus* is suggested as the first lineage to diverge (PP: 0.85), ML analysis of dataset 1, as well as ML and Bayesian

analyses of dataset 2 indicates a *Theropithecus* + *Papio* clade to the exclusion of *Lophocebus*. Node supports for respective branching patterns are low (dataset 1, BP: 50%; dataset 2, PP: 0.89; BP: 83%). Similarly, the Densitree indicates *Lophocebus* + *Papio* as the most frequent clade, while the second most frequent clade is formed by *Theropithecus* and *Papio*. Estimated divergence ages suggest that respective splitting events occurred during a short time period around 5 Ma. Among *Papio* representatives the tree topology is identical and divergence ages are similar as previously reported [54], depicting paraphyletic relationships in *P. ursinus, P. cynocephalus* and *P. hamadryas*, and polyphyletic relationships in *P. anubis*. According to estimated divergence ages, splitting events within *Papio* started around 2 Ma. Among macaques, *Macaca sylvanus* diverged first, 5.9-6.3 Ma. Subsequently the Asian macaques radiated and successively split up into the six Asian species groups. The *M. silenus* + *M. tonkeana* (*M. tonkeana* as representative of the Sulawesi group) clade separated from the remaining macaques between 5.2-5.9 Ma and further segregated into two species groups (3.2-4.6 Ma). Among the remaining macaques, *M. thibetana* (as representative of the *M. sinica* group) diverged between 3.9-5.0 Ma from a *M. fascicularis* + *M. arctoides* + *M. mulatta* clade. Within the latter, *M. fascicularis* split off first (3.2-4.6 Ma) whereas *M. arctoides* separated from the *M. mulatta* clade slightly later (2.7-4.3 Ma). Within *M. fascicluaris* and *M. mulatta* we found relatively ancient splitting events of 1.1-2.2 Ma and 1.4-2.9 Ma.

Discussion

The application of complete mtDNA genome sequences revealed highly supported branching patterns for most of the investigated papionin lineages. The mtDNA gene tree as well as estimated divergence ages are broadly consistent with those reported in previous studies, but also show some remarkable, but not unexpected discordances to recent nDNA studies [15,19,20,54,64,65].

The major findings of our analysis are: 1) a sister grouping of *Macaca* and the *Mandrillus* + *Cercocebus* clade, 2) paraphyly within the *Mandrillus* + *Cercocebus* clade, 3) unresolved relationships among *Papio*, *Lophocebus* and *Theropithecus*, and 4) similar divergence ages among *Macaca* species groups and papioninan genera. Furthermore, our phylogenetic reconstruction reveals highly supported branching patterns among the seven *Macaca* species groups, which are largely in

agreement with most previous studies, (e.g. [15,37,66]). The only exception is the phylogenetic position of *M. arctoides*, which is here strongly supported as the sister lineage to the *M. mulatta* group. This finding is not surprising given the evidence that *M. arctoides* is the result of hybridization between ancestral forms of the *M. sinica* and *M. mulatta* groups [37,66].

Divergence dates are mostly consistent regardless of the software (BEAST or PhyloBayes) and clock model (auto-correlated or uncorrelated) that were applied (Additional file 2: Table S1, Additional file 5: Figure S3, Additional file 6: Figure S4). Our estimation indicates a separation of African and Asian macaques around 6 Ma which is in line with Alba et al. [27], who, based on fossil data, proposed a macaque dispersal from Africa into Eurasia by the Late Miocene (5.3-5.9 Ma). Generally, our divergence age estimations reveal a stepwise but rapid radiation of macaque genera between 5.9 and 2.7 Ma in Asia, which is in agreement with the appearance of the earliest *Macaca*-like fossil in Asia which was found in the Yushe Basin (China) from about 4 Ma [27]. At that time two of the six main lineages of Asian macaques were already established as indicated by our divergence age estimations. To further test possible dispersal scenarios in Southeast Asia and especially in Sundaland further taxa of the species groups from different locations have to be included in future analyses.

We found the *Mandrillus* + *Cercocebus* clade to be more closely related to the macaques than to other African Papionina, a pattern also reported by Finstermeier et al. [19] and Pozzi et al. [20]. However, in contrast to Finstermeier et al. [19] alternative tree topology tests with our data were clearly rejected (Figure 3), which most likely can be explained by the increased taxon sampling in our study (33 sequences this study, 11 sequences in Finstermeier et al. [19]), because it is known to reduce phylogenetic error [67-70]. Moreover, since we controlled for the observed shift in A/C content, the *Mandrillus* + *Cercocebus* clade might be indeed more closely related to *Macaca* than to the other African papionins, at least if we consider mtDNA. This finding, however, is contradictory to relationships based on recent nuclear studies, which found the Macacina and Papionina to be reciprocally monophyletic [15,18]. Perelman et al. [15] found this branching pattern in a concatenated dataset of 54 nDNA loci (BP: 100%) as well as in six separately analysed subsets, of which four are similarly highly supported (BP: 97-100%). Likewise, the presence/absence

pattern of Alu integrations revealed no conflicting integrations, suggesting reciprocal monophyly of both clades [18] and Springer et al. [71], analysing a combined dataset of mtDNA and nDNA sequences, found the same pattern. Interestingly, comparative morphological studies investigating postcranial traits of African Papionina (*Mandrillus*, *Cercocebus*, *Lophocebus* and *Papio*) and one species of *Macaca* (*M. nemestrina*) suggest some similarities between *Mandrillus* + *Cercocebus* and the macaque [45,55]. However, since only one macaque species was included in the analysis, results concerning the relationship of *Mandrillus* + *Cercocebus* to *Macaca* have to be considered with caution. The question is whether the similarities between *Mandrillus*, *Cercocebus* and *M. nemestrina* are due to the plesiomorphy of the traits as suggested by Fleagle & McGraw [45,55] or whether they result from convergent adaptations to similar ecological niches since *Mandrillus*, *Cercocebus* and *M. nemestrina* are predominantly forest dwelling terrestrial primates [72,73]. Given that nDNA phylogenies (e.g. [15]) may reflect the true species relationships more reliably than mtDNA phylogenies with *Macaca* being basal to the Papionina, we would assume that morphological similarities result from convergent adaptation. In contrast, the present mtDNA phylogeny would rather accord to the assumption that the shared morphological features are primitive.

Inconsistencies of mitochondrial and nuclear phylogenies are often explained by incomplete lineage sorting or ancient hybridization [5,19,37,59,60,74,75]. At the moment, we cannot determine if one or both phenomena affected the suggested phylogenetic relationships. A possible scenario based on hybridization could be that ancestral representatives of the *Mandrillus* + *Cercocebus* clade were indeed more closely related to ancestral macaques, but were later introgressed by an ancestor of the *Papio* + *Theropithecus* + *Lophocebus* clade, resulting in nuclear swamping. Hybridization seems to be common among extant papioninan taxa, even between genera [11,12,76,77]. It is therefore likely that hybridization and introgression also occurred among the ancestral papioninan lineages which lead to the observed incongruence between nDNA and mtDNA phylogenies. However, as mentioned above, incomplete sorting of mitochondrial lineages in these taxa is also a plausible explanation for the observed relationships.

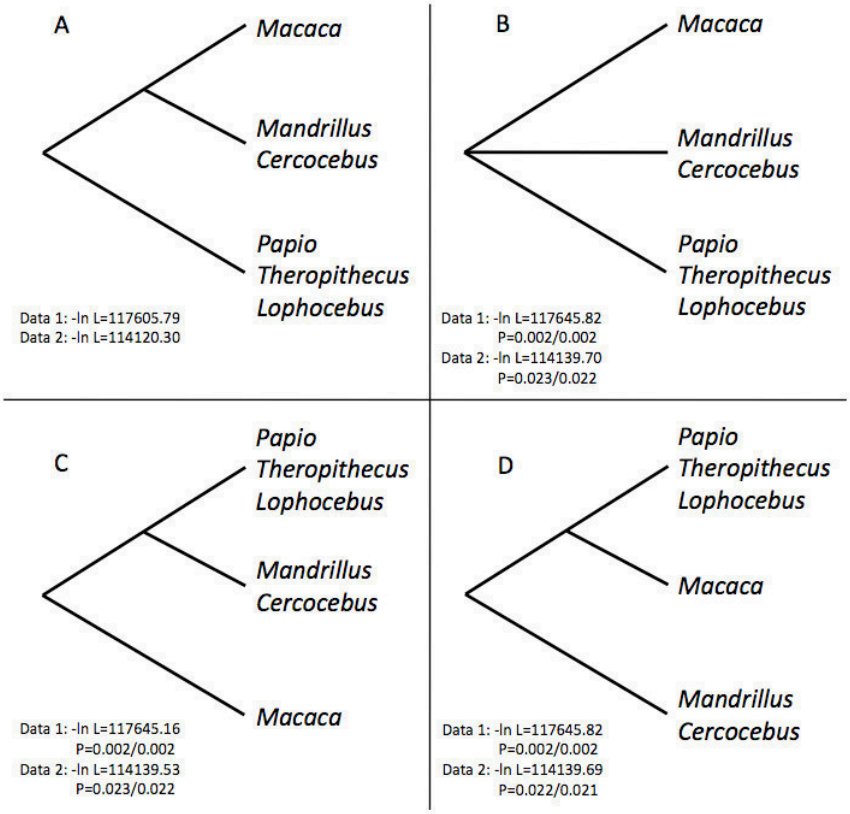

Figure 3 Tree topologies that were tested in the alternative tree topology test. Tree **A** represents the most probable topology, whereas **B**, **C** and **D** were significantly rejected. Log-likelihood and P values for each tree topology are given for dataset 1 and 2, respectively. First and second P values resulted from the Kishino-Hasegawa and the Shimodaira-Hasegawa tests, respectively.

Our mtDNA genome tree revealed paraphyletic relationships of *Mandrillus* and *Cercocebus* taxa, which is again contradictory to nDNA studies that suggest both genera to be reciprocally monophyletic [14,15]. As our data show, *M. leucophaeus* clusters with *C. chrysogaster* and *M. sphinx* is indicated as sister lineage to both to the exclusion of *C. torquatus* and *C. atys*. Again, ancient hybridization and incomplete lineage sorting cannot be excluded as having affected this branching pattern. However, since the species identification of the herein used *C. torquatus* sample is questionable (originally identified as *Lophocebus albigena* [78]), our results have to be regarded as preliminary and at the moment any further discussion of possible phylogeographic scenarios would remain highly speculative. Interestingly, however, the sister relationship of *C. chrysogaster* to *M. leucophaeus* is consistent with Kingdon's [79] p.46 observation that *C. chrysogaster* is morphologically "the

most drill-like of the drill-mangabeys". On the other hand, Kingdon´s suggestion has not been held up by several other studies, which find *C. torquatus* to be the most primitive and *Mandrillus*-like mangabey [14,45,46,55,72]. Comprehensive sampling of mangabeys with reliable information on their geographic provenance is required to further elucidate relationships within the *Mandrillus* + *Cercocebus* clade.

Relationships among *Papio*, *Theropithecus* and *Lophocebus* have been analysed in several studies, but differed depending on the markers that were applied. Chatterjee at al. [56] investigated seven mitochondrial genes and found *Theropithecus* clustering with *Lophocebus* to the exclusion of *Papio* while Finstermeier et al. [19] showed a closer, but only weakly supported mtDNA genome affiliation of *Papio* to *Theropithecus*; Pozzi et al. [20] were also not able to resolve these relationships. Likewise, while we found *Theropithecus* split off first in the Bayesian analysis of the original dataset, ML analysis as well as both, Bayesian and ML estimations of dataset 2 suggested *Lophocebus* in the basal position. For both datasets, support values for respective branching patterns are low and estimated divergence ages among the three genera indicate a rapid radiation around 5 Ma. Also in the Densitree, different branching patterns are depicted. Accordingly, the present data are probably not sufficient to resolve the branching pattern. On the other hand, nDNA sequence data revealed a more consistent picture by placing *Lophocebus* with *Papio* to the exclusion of *Theropithecus* [14,15,48,56,71]. Not surprisingly, morphological (i.e., craniodental) data are congruent with these molecular studies when allometry is properly accounted [80,81]. Guevara & Steiper [14] stated that the basal position of *Theropithecus* is plausible given that known fossils [82] of the genus are considerably older (~4.0 Ma) than that of *Papio* (~2.5 Ma) and *Lophocebus* (~2.0 Ma). It has been shown that an increased sampling of more individuals per species may help to resolve phylogenies with short internodes, but nevertheless an increased sampling will not improve the phylogenies when hybridisation has confounded it [14,74].

The initial radiation within the Papionini into the three main lineages 1) *Papio*, *Theropithecus* and *Lophocebus*, 2) *Mandrillus* and *Cercocebus*, and 3) *Macaca* took place during the Late Miocene. Within these three clades, further differentiation events occurred on similar time scales (*Theropithecus* – *Lophocebus* – *Papio*: 5–6 Ma; *Mandrillus* – *Cercocebus*: 4–5 Ma; *Macaca*: 5–6 Ma). (Figure 1, Additional file 2:

Table S1, Additional file 4: Figure S2). This means that, although macaques seem morphologically not as diverse as their African sister taxa [23,35,83], the mitochondrial heterogeneity among species groups is at least as high as among the African papionin genera. Comparing our mtDNA divergence ages with those inferred from nDNA data (e.g. [15]) we find that those splits slightly differ but tend to be in the same range (Additional file 2: Table S1). We therefore can assume nuclear heterogeneity among *Macaca* species groups and Papionina genera to be also similar.

Given the equally long independent evolutionary histories of macaque species groups and Papionina genera the question of whether the species groups represent rather distinct genera or whether the two main African Papionina clades constitute only two genera (*Papio* and *Cercocebus*) with diverse species groups seems a subject for debate. However, due to morphological similarities of the macaque taxa and the morphological differences between the African genera, a reorganisation of their taxonomic ranks based on time depths as proposed by Goodman [84] and Groves [23,85] seems not to be justified at the moment.

Conclusion

By analysing complete mtDNA genomes of all papionin genera (with the exception of *Rungwecebus*) we obtained well-resolved phylogenetic relationships and higher support values than inferred from shorter mtDNA fragments. Our estimated divergence ages are similar to those of other studies but credibility intervals are narrowed down due to the application of complete mtDNA genome sequences. Including an increased number of papionin samples led to a different tree topology concerning the phylogenetic position of the *Mandrillus* + *Cercocebus* clade among papionins, which is in stark contrast to previous nDNA studies, indicating that ancient introgression or incomplete lineage sorting may play a role here. However, which of the two processes led to these contradictions cannot be determined here since we analysed only the maternal lineage of included taxa.

Although the mtDNA tree is just a single gene tree, it offers important additional information on the evolutionary history of the Papionini. Future investigations should incorporate a large number of nDNA loci or even complete genome data to possibly

distinguish introgression or incomplete lineage sorting. Furthermore, for a reliable comparative study of mtDNA and nDNA sequences data, respective loci are at best obtained from the same individuals or at least the same species. Since respective nDNA data is by now not available from GenBank we focused solely on mtDNA data. In addition to nDNA data future studies should also include comprehensive sequence data of the herein unstudied genus *Rungwecebus*. There is also a need to further elucidate intra-generic taxonomy and phylogeny in almost all papionin genera, particularly in *Cercocebus*. Therefore special attention must be paid to the geographic provenance of studied samples.

Methods

Sample collection

Blood samples from one individual each of *M. arctoides* (*M. arctoides* group), *M. silenus* (*M. silenus* group), *M. tonkeana* (Sulawesi group), *M. fascicularis* (*M. fascicularis* group) and *M. sylvanus* (*M. sylvanus* group), and two individuals of *M. mulatta* (*M. mulatta* group) were obtained from European zoos, Covance and the German Primate Center. All blood samples were taken during routine health checks by experienced veterinarians and not specifically for this study. A fresh tissue sample from a deceased *M. thibetana* (*M. sinica* group) individual was obtained from the Strasbourg Primate Center. Sample collection was approved by the Animal Welfare Body of the German Primate Center and adhered to the American Society of Primatologists Principles for the Ethical Treatment of Non-Human Primates (see www.asp.org/ society/policy.cfm). No animals were sacrificed for this study.

Laboratory methods

Genomic DNA from blood and tissue samples was extracted using the Qiagen DNeasy Blood & Tissue Kit following the supplier's recommendations. To minimize the chance of amplifying nuclear mitochondrial-like sequences (numts) [86], two overlapping long-range PCR fragments were generated (8 kb and 10 kb) using primers specifically designed for macaque species groups on the basis of available sequence data in GenBank and the Long Range dNTPack from Roche. Conditions for the long-range PCR amplification comprised a pre-denaturation step at 94°C for 2

min, followed by 40 cycles at 94°C for 1 min, annealing at 60°C for 1 min and extension at 68°C for 20 min. At the end a final extension step at 68°C for 30 min was added. PCR products were visualized on 1% agarose gel and extracted with the Qiagen PCR purification Kit. Obtained long-range fragments were used as template for nested PCRs to generate products of 1.0 to 1.2 kb. Respective primers are available from the authors upon request. PCR conditions for nested PCRs comprised a pre-denaturation step at 94°C for 2 min, followed by 40 cycles each with denaturation at 94°C for 1 min, annealing at 60°C for 1 min and extension at 72°C for 1.5 min, and terminating with a final extension step at 72°C for 5 min. PCR products were again checked on 1% agarose gels, and subsequently extracted and sequenced on an ABI 3130*xL* sequencer using the BigDye Terminator Cycle Sequencing Kit (Applied Biosystems) and the amplification primers. DNA extraction, PCR set-up, gel extraction and sequencing were performed in separate laboratories. Genome sequences were assembled with SeaView 4.4.0. [87] and annotation was conducted with the online program DOGMA [88] and manually checked. Sequences in the overlapping parts of the two long-range PCRs were identical and all protein-coding genes were correctly translated without any premature stop codons, indicating that no numt contamination is present in our data. All sequences were deposited at GenBank (for accession numbers see Additional file 7: Table S2).

Data analysis

The dataset for the phylogenetic analysis comprised a total of 38 mtDNA genome sequences including 13 macaques representing all seven species groups (2 *M. sylvanus*, 1 *M. silenus*, 1 *M. tonkeana*, 2 *M. thibetana*, 3 *M. mulatta*, 3 *M. fascicularis* and 1 *M. arctoides*), eleven baboons (2 *P. ursinus*, 2 *P. hamadryas*, 3 *P. anubis*, 2 *P. cynocephalus*, 1 *P. kindae* and 1 *P. papio*), three geladas (*T. gelada*), one drill (*M. leucophaeus*), one mandrill (*M. sphinx*), one crested mangabey (*L. aterrimus*), three capped mangabeys (1 *C. chrysogaster*, 1 *C. atys*, 1 *C. torquatus*) and five non-papionin primate species (*Chlorocebus pygerythrus, Colobus guereza, Pongo abelii, Pan troglodytes, Homo sapiens*). Accordingly, *Rungwecebus* was the only missing papionin genus. The identity of the *C. torquatus* individual remained ambiguous. While it was originally assigned to *Lophocebus albigena* [78], BLAST-search revealed that it is 99-100% identical to available mtDNA sequences of *C. torquatus*.

For information about GenBank accession numbers and the source of the herein used sequences see Additional file 7: Table S2.

Sequences were aligned with Muscle 3.7 [89] as implemented in SeaView and manually corrected. For phylogenetic tree reconstructions, indels and poorly aligned positions were removed with Gblocks 0.91b [90]. To check for possible shifts in base composition among species, we calculated the base composition for each species using PAUP 4.0b10 [91]. Since we observed a slight shift in A/C content among papionins (Additional file 3: Figure S1) and to test whether this shift might have influenced phylogenetic relationships, we generated a second alignment (dataset 2) in which positions that contained both an Adenin and Cytosin were masked with an "M" (in total 606 positions).

The programs RAxML 0.93 [92] and MrBayes 3.1.2 [93,94] were used for phylogenetic tree reconstructions applying ML and Bayesian algorithms. As substitution models for Bayesian reconstructions we applied the TrN + I + G and GTR + I + G models for datasets 1 and 2, respectively, as they were selected as best-fit models by jModeltest 2.1 [95] under the Bayesian information criterion (BIC) and the Decision Theory Performance-based Selection (DT). In MrBayes we analysed four independent Markov Chain Monte Carlo (MCMC) runs with a default temperature of 0.2. All repetitions were run for 1 million generations with tree and parameter sampling setting in every 100 generations. The first 25% of samples were discarded as burn-in, resulting in 75,001 trees per run. The adequacy of the burn-in and convergence of all parameters was assessed via the uncorrected potential scale reduction factor (PSRF) [96] as calculated by MrBayes and by visual inspection of the trace of the parameters across generations using the software TRACER 1.5 [97]. To check whether posterior clade probabilities were also converging, AWTY [98] was used. Posterior probabilities for each split and a phylogram with mean branch lengths were calculated from the posterior density of trees. Both ML calculations in RAxML were run with the CAT-GTR model and 1,000 rapid bootstrapping replications. Alternative phylogenetic relationships among the three observed major papionin clades were tested with the Kishino-Hasegawa test [99] and Shimodaira-Hasegawa test [100] with full optimisation and 1,000 bootstrap replications in PAUP.

Divergence ages were estimated applying both, uncorrelated and auto-correlated, clock models. To calculate divergence ages with an uncorrelated clock model, we used BEAST 1.6.1 [101,102]. We assumed a relaxed lognormal model of lineage variation and a Birth-Death Process prior for branching rates. In contrast to Finstermeier et al. [19], branching of *Mandrillus* + *Cercocebus* with *Macaca* was not constrained in our study as alternative branching patterns were rejected by alternative tree topology tests.

The following five fossil-based calibration points were applied with a normal distribution prior for respective nodes: The *Homo* – *Pan* split 6.5 Ma with a 95% credibility interval (CI) of 0.5 Ma [103-105]. The split between *Pongo* and the *Homo-Pan* lineage at 14.0 Ma (95% CI: 1.0 Ma) [106], the divergence of *Theropithecus* and *Papio* 5.0 Ma (95% CI: 1.5 Ma) [107,108], the split between African and Asian macaques at 5.5 Ma (95% CI: 1.0 Ma) [27,108] and the separation of hominoids and cercopithecoids at 27.5 Ma (95% CI: 3.5 Ma) [109-111].

In total, we ran four replicates in BEAST, each with 25 million generations, and tree and parameter sampling every 1,000 generations. TRACER was applied to assess the adequacy of a 10% burn-in and the convergence. The sampling distributions were combined (25% burn-in) with LogCombiner 1.6.1 and a consensus chronogram with node height distribution was generated and visualized with TreeAnnotator 1.6.1 and FigTree 1.4.0 [112].

To see whether the application of an auto-correlated model instead of an uncorrelated model has an effect on the divergence time estimation we performed Bayesian molecular dating with the software package PhyloBayes 3.3 [113]. The tree topology was fixed using the topology as inferred from MrBayes. Five node ages were fixed by specifying calibration intervals based on the same calibration points and credibility interval as mention above. In the main program of PhyloBayes (pb) the CAT-GTR model was applied in combination with a log-normal auto-correlated (−ln) [114] relaxed clock model and in a second independent run with an uncorrelated (−ugam) [101] relaxed clock model. We monitored the development of the log-likelihood as a function of time and found it to be stable (to show convergence) after approximately 3,000–4,000 cycles. Hence, 10,000 cycles were carried out discarding the first 2,500 trees as burn-in. A posterior consensus

chronogram was calculated on the remaining 7,500 trees using the post analysis program readpb and was visualized with Figtree.

Availability of supporting data

The data set supporting the results of this article is available in the Data Dryad repository, DOI: 10.5061/dryad.9tm42

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

RL did laboratory work, analysed data, and wrote the paper. MB analysed data. DZ and CR designed the study, analysed data, and wrote the paper. All authors read and approved the final manuscript.

Acknowledgements

We thank the zoos in Dresden, Madrid, Salem, Straubing and Wuppertal as well as Covance Inc. (Münster, Gemany), the Strasbourg Primate Center and the German Primate Center for providing valuable macaque samples. We are also grateful to Christiane Schwarz for her excellent laboratory work, and Colin Groves and Brandon C. Wheeler for their valuable comments on an earlier version of the paper and their corrections of the English. We thank the editor and three anonymous reviewers for critical comments on an earlier version of the manuscript.

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Chapter 3

AMERICAN JOURNAL OF PHYSICAL ANTHROPOLOGY 000:000–000 (2012)

Baboon Phylogeny as Inferred From Complete Mitochondrial Genomes

Dietmar Zinner,[1]* Jenny Wertheimer,[1,2] Rasmus Liedigk,[2] Linn F. Groeneveld,[3] and Christian Roos[2,4]

[1]*Cognitive Ethology Laboratory, German Primate Center, 37077 Göttingen, Germany*
[2]*Primate Genetics Laboratory, German Primate Center, 37077 Göttingen, Germany*
[3]*Courant Research Centre Geobiology, Georg-August-Universität Göttingen, 37077 Göttingen, Germany*
[4]*Gene Bank of Primates, German Primate Center, 37077 Göttingen, Germany*

KEY WORDS *Papio*; evolution; Africa

ABSTRACT Baboons (genus *Papio*) are an interesting phylogeographical primate model for the evolution of savanna species during the Pleistocene. Earlier studies, based on partial mitochondrial sequence information, revealed seven major haplogroups indicating multiple para- and polyphylies among the six baboon species. The most basal splits among baboon lineages remained unresolved and the credibility intervals for divergence time estimates were rather large. Assuming that genetic variation within the two studied mitochondrial loci so far was insufficient to infer the apparently rapid early radiation of baboons we used complete mitochondrial sequence information of ten specimens, representing all major baboon lineages, to reconstruct a baboon phylogeny and to re-estimate divergence times. Our data confirmed the earlier tree topology including the para- and polyphyletic relationships of most baboon species; divergence time estimates are slightly younger and credibility intervals narrowed substantially, thus making the estimates more precise. However, the most basal relationships could not be resolved and it remains open whether (1) the most southern population of baboons diverged first or (2) a major split occurred between southern and northern clades. Our study shows that complete mitochondrial genome sequences are more effective to reconstruct robust phylogenies and to narrow down estimated divergence time intervals than only short portions of the mitochondrial genome, although there are also limitations in resolving phylogenetic relationships. Am J Phys Anthropol 000:000–000, 2012.

Baboons of the genus *Papio* have been regarded as a useful analog for hominin behavioral and biological evolution because their evolutionary history took place in parallel to hominins in similar African savanna habitats (Jolly, 1970, 2001; Strum and Mitchell, 1987; Elton, 2006; Codron et al., 2008; Swedell and Plummer, 2012). Extant baboons occur in large parts of sub-Saharan Africa outside the Central- and West African rainforests and are also found in Southwestern Arabia (Jolly, 1993; Kingdon, 1997; Groves, 2001) (Fig. 1a). Representatives of the genus *Papio* are traditionally divided into five different species or morphotypes, based on morphological, ecological, and behavioral characteristics (Hill, 1970). These are Guinea baboons (*P. papio*), olive baboons (*P. anubis*), hamadryas baboons (*P. hamadryas*), yellow baboons (*P. cynocephalus*), and chacma baboons (*P. ursinus*). A similar taxonomic status as for these five forms is warranted for Kinda baboons (*P. kindae*) (Jolly, 1993, 2001; Frost et al., 2003; Burell, 2008; Zinner et al., 2009). Whether these types should be classified as subspecies of the superspecies *P. hamadryas* (Jolly, 1993) or as distinct species (Groves, 2001; Grubb et al., 2003) is still disputed, but recent studies recognize the six morphotypes as species (Zinner et al., 2011a, 2012).

Various studies tried to clarify the phylogenetic relationships within *Papio* by analyzing parts of the mitochondrial genome, such as the "Brown region" (Brown et al., 1982) or the cytochrome *b* gene (Newman et al., 2004; Wildman et al., 2004; Zinner et al., 2009; Keller et al., 2010). Zinner et al. (2009) attempted to resolve the phylogenetic relationships of baboons using both the "Brown region" and the cytochrome *b* gene, and revealed seven well-supported major haplogroups (Fig. 1b). The study revealed paraphylies in all species, except for Guinea and Kinda baboons, due to discordances between mitochondrial phylogeny and morphology and/or geographic distribution. However, a strong geographical signal with haplotypes of parapatric populations from different species clustering together was found (Zinner et al., 2009). Although the seven major haplogroups were strongly supported, phylogenetic relationships among them remained largely unresolved. Moreover, divergence time estimates indicated a fast radiation-like (star-like) splitting event into various baboon lineages, starting ~ 2.09 million years ago (Ma) (Zinner et al., 2009), which might impede inferring the basal relationships with confidence.

It is plausible that genetic variation within the two studied mitochondrial loci was insufficient to infer the apparently rapid early radiation of baboons. The use of complete mitochondrial sequence information might

Additional Supporting Information may be found in the online version of this article.

Grant sponsor: German Primate Center.

*Correspondence to: Dietmar Zinner, Cognitive Ethology Laboratory, German Primate Center, Kellnerweg 4, 37077 Göttingen, Germany. E-mail: dzinner@gwdg.de

Received 26 June 2012; accepted 8 October 2012

DOI 10.1002/ajpa.22185
Published online in Wiley Online Library (wileyonlinelibrary.com).

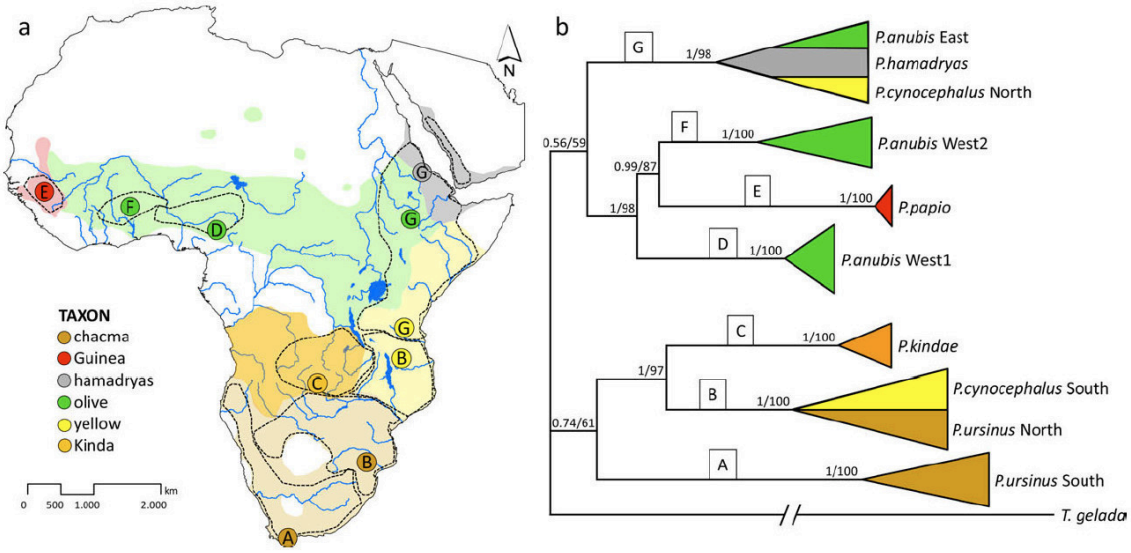

Fig. 1. (**a**) Distribution of the six baboon species and the seven major mitochondrial haplogroups (A–G) (map based on Kingdon, 1997; Jolly, 2007; Zinner et al., 2009, in press), and (**b**) simplified phylogenetic relationships among the haplogroups (adapted from Zinner et al., 2009). In (a), dashed lines and colored circles indicate the distribution of the seven major mitochondrial haplogroups and the geographic provenance of samples used in this study (see also Table 1), respectively. In (b), numbers on branches represent support values from Bayesian inference and maximum-likelihood analysis, respectively.

allow a better resolution and stronger statistical support in the phylogenetic tree reconstruction and to narrow down the divergence time credibility intervals (DeFilippis and Moore, 2000; Duchêne et al., 2011; Rokas and Carroll, 2005). A similar approach was successfully applied in recent phylogenetic studies of other taxonomic groups, e.g., gibbons (Chan et al., 2010), colobines (Roos et al., 2011; Liedigk et al., 2012), squirrel monkeys (Chiou et al., 2011); woodpeckers (DeFilippis and Moore, 2000), dolphins (Vilstrup et al., 2011), and bears (Yu et al., 2007). Hence, to test whether sequence information from the complete mitochondrial genome allows a better resolution of phylogenetic relationships and results in smaller divergence time credibility intervals than shorter mitochondrial fragments in baboons, we sequenced and analyzed complete mitochondrial genomes of ten baboons representing all six species and the seven major mitochondrial haplogroups, and compared them with published data.

MATERIALS AND METHODS

Sample collection

We used ten baboon fecal samples, which were collected at various sites in Africa (Fig. 1a and Table 1) for earlier phylogeographic studies (see Zinner et al., 2009 and Keller et al., 2010). We used two types of information to assign samples to respective species: (1) characteristic morphological cues and (2) biogeographic provenance of samples (see Zinner et al., 2009). While observing the animals directly in the field, we used pelage color, body size, general body form, carriage of the tail (curved or "broken") for species identification (after Kingdon, 1997). We further compared the appearance of the baboons in the field with pictures in Kingdon (1997). The ten baboon samples represent all six baboon species and the seven described haplogroups (Fig. 1b, Zinner et al., 2009): *P. ursinus* South (haplogroup A), *P. ursinus* North, and *P. cynocephalus* South (haplogroup B), *P. kindae* (haplogroup C), *P. anubis* West1 (haplogroup D), *P. papio* (haplogroup E), *P. anubis* West2 (haplogroup F), and *P. anubis* East, *P. hamadryas* and *P. cynocephalus* North (haplogroup G). The respective samples were randomly selected from collections of samples of the respective species and haplogroups used in an earlier analysis (Zinner et al., 2009). Fresh fecal material was preserved in 40 ml 75% ethanol and dry samples were stored directly on 40 ml silica gel in 50-ml tubes. We stored the samples at ambient temperature for up to 6 months before further processing. The geographic coordinates of the sampling locations were recorded with a GPS.

Our study complied with protocols approved by the respective authorities in countries of origin, and adhered to the legal requirements of the countries in which research was conducted. The study was carried out in compliance with respective animal care regulations and the principles of the American Society of Primatologists and the German Primate Center for the ethical treatment of nonhuman primates.

Laboratory methods

DNA from fecal material was extracted using the QIAamp DNA Stool Mini Kit (Qiagen, Germany) following manufacturer's protocol with some modifications (Yang et al., 2012). Because of degradation of the DNA extracted from feces, mitochondrial genomes were amplified via 5–25 overlapping fragments, and nested PCRs with an average length of 1,000 bp were sequenced on an ABI 3130*xL* sequencer. Respective laboratory methods are outlined in detail in Roos et al. (2011) and Liedigk et al. (2012). To prevent crossindividual contamination, laboratory procedures followed described standards (Roos et al., 2008). Accordingly, DNA extraction,

TABLE 1. Baboon samples

Species	Haplogroup	Site	Country	Long	Lat	Acc no
P. anubis	G	Managasha NP	Ethiopia	38.57125	8.96838	JX946196
P. anubis	D	Komoe NP	Côte d'Ivoire	−3.79000	8.80000	JX946198
P. anubis	F	Gashaka-Gumti NP	Nigeria	11.58333	7.31667	JX946197
P. cynocephalus	G	Mikumi NP	Tanzania	37.16463	−7.34651	JX946199
P. cynocephalus	B	Amani	Tanzania	37.51363	−11.26054	JX946200
P. hamadryas	G	Furrus	Eritrea	38.97115	15.01148	JX946201
P. papio	E	Niokolo Koba NP	Senegal	−12.76667	12.88333	JX946203
P. kindae	C	Kasanka NP	Zambia	30.25202	−12.59059	JX946202
P. ursinus	B	DeHoop NR	South Africa	20.40658	−34.45621	JX946204
P. ursinus	A	Blyde River	South Africa	30.79000	−24.68000	JX946205

NP: national park; NR: nature reserve.
Species, haplogroup affiliation, geographical location and GenBank accession numbers of studied baboons.

PCR, gel extraction, and sequencing were performed in separate laboratories and randomly repeated after several months, while always only one individual was tested. Sequences from independent analyses were identical. Because only fecal material was used, a contamination of the dataset with nuclear integrations of mitochondrial fragments ("numts") can be regarded as minimal, because nuclear DNA is highly degraded in feces (Thalmann et al., 2004). In fact, test amplifications of the autosomal intron 3 of the serum albumin gene (ALB3) revealed positive PCR amplifications of only 200–400 bp from the 10 baboon DNAs. Further, no multiple amplifications of different copies were detected by direct sequencing of PCR products and overlapping fragments were identical as revealed by visually inspecting electropherograms of all sequences. Complete mitochondrial genome sequences were assembled with GeneiousPro 5.4 (Drummond et al., 2011) and annotated with the online program DOGMA (Wyman et al., 2004). According to DOGMA and manual verification, all protein-coding genes are correctly transcribed, and rRNAs and tRNAs are able to form their typical secondary structure. Sequences were deposited in GenBank (for accession numbers see Table 1).

Statistical analysis

For phylogenetic analysis, additional orthologous sequences of other primate taxa deposited in GenBank were added. The final dataset comprised 18 sequences including ten baboons, four other cercopithecines (*Theropithecus gelada* [FJ785426], *Macaca sylvanus* [AJ309865], *M. mulatta* [JQ821843], *Chlorocebus aethiops* [AY863426]), one colobine (*Colobus guereza* [AY863427]), and three hominoid species (*Homo sapiens* [X93334], *Pan troglodytes* [D38113], *Pongo abelii* [X97707]), that were used as outgroup taxa. Sequences were aligned with Muscle 3.7 (Edgar, 2004) and manually corrected. For phylogenetic analyses, two different datasets were generated. The first dataset (mtDNA1) consists of the complete mitochondrial genome in which only poorly aligned positions and indels were removed with Gblocks 0.91b (Castresana, 2000) using default settings. The second dataset (mtDNA2), generated in Mesquite 2.75 (Maddison and Maddison, 2011), included only the 12 protein-coding genes on the heavy strand.

Phylogenetic tree reconstructions were conducted with maximum-likelihood (ML) and Bayesian algorithms, using respectively the programs GARLI 2.0 (Zwickl, 2006) and MrBayes 3.1.2 (Huelsenbeck et al., 2001; Ronquist and Huelsenbeck, 2003). For all reconstructions, the best-fit model of nucleotide substitution was chosen with the Bayesian information criterion (BIC) in jModeltest 2.1 (Posada, 2009) (Supporting Information Table S1). For the mtDNA2 dataset, each locus was treated separately and each with its own substitution model. For ML reconstructions in GARLI, only the models were specified, while all other settings were left at their default value. Respective internal node support was assessed by bootstrap analyses with 500 replicates and majority-rule consensus trees were calculated in PAUP* 4.0b10 (Swofford, 2003). For Bayesian analyses, we applied four independent Markov Chain Monte Carlo (MCMC) runs with the default temperature of 0.2. We ran four repetitions for 10 million generations with tree and parameter sampling every 100 generations. Acceptance rates were in the optimal range of 10–70%. The adequacy of a 25% burn-in and convergence of all parameters was checked via the uncorrected potential scale reduction factor (PSRF) (Gelman and Rubin, 1992) as estimated by MrBayes and by inspecting the trace of the parameters across generations using the software TRACER 1.5 (Rambaut and Drummond, 2007). Whether posterior split probabilities were also converging was examined with AWTY (Nylander et al., 2008). Posterior probabilities and a phylogram with mean branch lengths were calculated from the posterior density of trees. Alternative phylogenetic positions of the *P. ursinus* South haplogroup among baboons, and various alternative relationships among the *P. anubis* West1, *P. anubis* West2 and *P. papio* haplogroups were evaluated with the Kishino–Hasegawa (Kishino and Hasegawa, 1989) and Shimodaira–Hasegawa (Shimodaira and Hasegawa, 1999) tests with full optimization and 1,000 bootstrap replicates in PAUP*.

Divergence ages from both mitochondrial datasets were estimated in BEAST 1.6.1 (Drummond and Rambaut, 2007) with a relaxed molecular clock approach (Drummond et al., 2006). Therefore, we assumed a relaxed lognormal model of lineage variation and a birth–death process prior for branching rates. The mtDNA2 dataset was partitioned treating each locus separately and each with its own substitution model, while dataset mtDNA1 was regarded as one partition. Five fossil-based calibration points were applied with a normal distribution prior for respective nodes: the split between *Homo* and *Pan* 6.5 Ma with a 95% credibility interval (CI) of 0.5 Ma (Vignaud et al., 2002; Brunet et al., 2005; Lebatard et al., 2008), the separation of *Pongo* from the *Homo* + *Pan* lineage 14 Ma and a 95% CI of 1.0 Ma (Kelley, 2002), the split between *Theropithecus* and *Papio* 4 Ma (95% CI: 0.5 Ma) (Leakey, 1993;

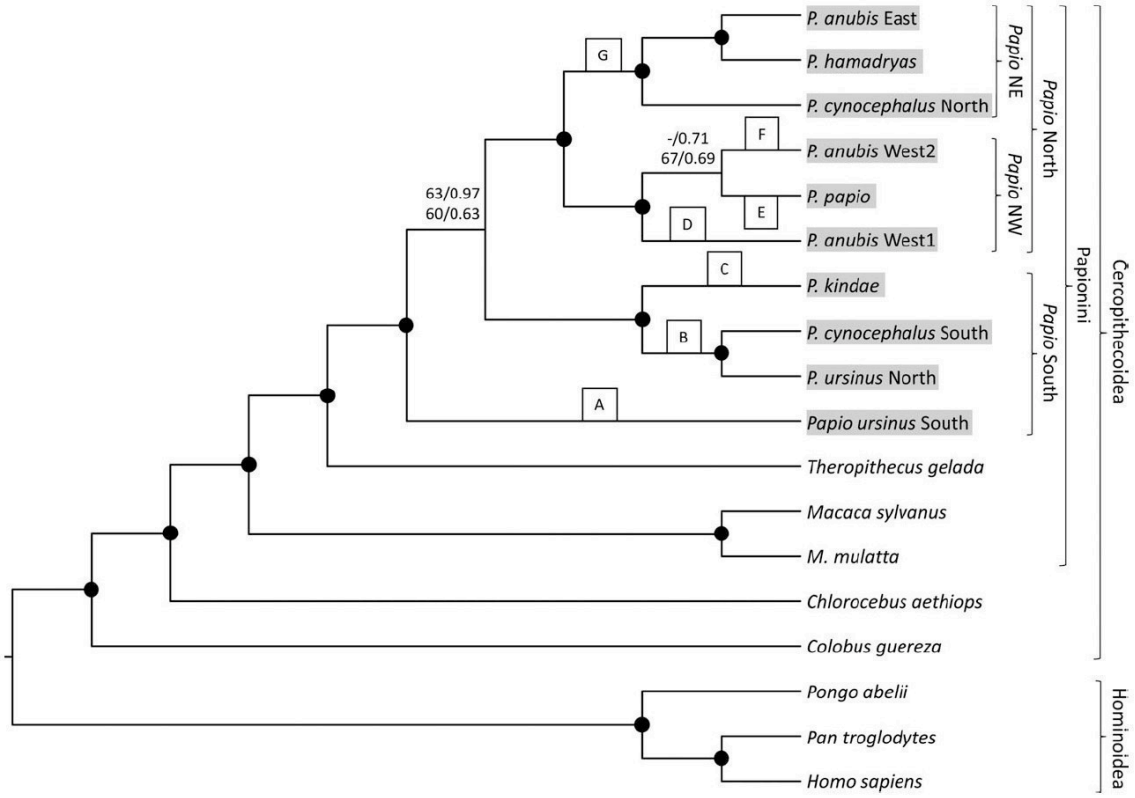

Fig. 2. Phylogenetic relationships among baboons and outgroup taxa based on complete mitochondrial genome sequences. Black dots on nodes indicate ML bootstrap values of 99–100% and Bayesian posterior probabilities of 1.0; values below are given at respective branches (upper line: mtDNA1, lower line: mtDNA2). **A**–**G** indicates the seven major haplogroups in *Papio* according to Zinner et al. (2009).

Delson, 2000), the split between *M. sylvanus* and *M. mulatta* 5.5 Ma (95% CI: 0.5 Ma) (Delson, 1980), and the divergence of hominoids and cercopithecoids 26.5 Ma (95% CI: 2.5 Ma) (Zalmout et al., 2010; Pozzi et al., 2011). We ran four replicates for 25 million generations with tree and parameter sampling occurring every 1,000 generations. TRACER was used to assess the adequacy of a 10% burn-in and convergence of all parameters across generations. Subsequently, sampling distributions were combined (25% burn-in) with the software LogCombiner 1.6.1 and a consensus chronogram with node height distribution was generated and visualized with TreeAnnotator 1.6.1 and FigTree 1.3.1 (Rambaut, 2008).

RESULTS

Mitochondrial genome sequences were successfully amplified and sequenced from ten *Papio* individuals. All sequenced genomes consisted of 13 protein-coding genes, 2 rRNA genes, 22 tRNA genes, and the control region. The initial alignment comprising a total of 18 sequences had a length of 16,858 bp. After removing poorly aligned positions and indels, the mtDNA1 dataset had a length of 16,055 bp, while the mtDNA2 dataset, including only the 12 protein-coding genes on the heavy strand, was 10,854 bp in length.

Phylogenetic trees obtained from both datasets (mtDNA1 and mtDNA2) and derived from Bayesian inference and the ML algorithm yielded identical tree topologies and mainly well supported branching patterns (ML bootstrap values: 99–100%, Bayesian posterior probabilities: 1.0) (Fig. 2). The only exceptions are the phylogenetic position of *P. ursinus* South, and the relationships among the two western *P. anubis* and *P. papio*. According to our tree reconstructions, *P. ursinus* South diverged first and appears as sister lineage to all other baboons. However, statistical support is weak (ML bootstrap values: 60–63%, Bayesian posterior probabilities: 0.63–0.97) and alternative positions of *P. ursinus* South within the baboon clade (sister to either the southern or northern clades, or an unresolved trichotomy among these two clades and *P. ursinus* South) are statistically not rejected ($P > 0.05$). After this initial split, a major division occurred between the remaining southern and the northern lineages. Among the southern lineages, *P. kindae* split off before *P. ursinus* North and *P. cynocephalus* South separated. The northern lineages first divided into a northwestern and a northeastern clade. Within the latter, *P. cynocephalus* North diverged first from a clade consisting of *P. anubis* East and *P. hamadryas*. In the northwestern clade, *P. anubis* West1 is suggested to be the sister lineage to the *P. anubis* West2—*P. papio* clade. However, statistical support for this relationship is weak (ML bootstrap values: <50–67%, Bayesian posterior probabilities: 0.69–0.71) and alternative relationships (*P. anubis* West1 and West2 are sister taxa,

TABLE 2. Estimation of divergence ages in Ma (95% credibility intervals)

Split	mtDNA1 divergence ages	mtDNA2 divergence ages	Brown + cyt b divergence ages
Cercopithecoidea − Hominoidea	26.66 (24.29–28.95)	27.13 (24.75–29.57)	24.38 (18.98–30.33)
Pongo − *Homo* + *Pan*	13.61 (12.54–14.73)	13.74 (12.71–14.84)	13.74 (12.59–14.90)
Homo – *Pan*	6.18 (5.60–6.75)	6.19 (5.59–6.78)	6.43 (5.85–7.01)
Colobus – Cercopithecinae	17.94 (15.21–20.59)	19.05 (16.13–22.20)	15.63 (11.50–20.08)
Chlorocebus – Papionini	12.08 (10.38–13.82)	12.34 (10.54–14.11)	9.80 (7.72–12.07)
Macaca − *Papio* + *Theropithecus*	10.22 (8.73–11.73)	10.09 (8.62–11.55)	7.41 (6.42–8.46)
Macaca sylvanus − *M. mulatta*	5.54 (4.98–6.06)	5.51 (4.94–6.03)	4.75 (3.27–6.29)
Papio – *Theropithecus*	4.54 (4.04–5.04)	4.36 (3.87–4.86)	3.99 (2.92–5.09)
P. ursinus South − remaining baboons	2.21 (1.91–2.53)	1.96 (1.68–2.28)	
Papio southern clades – *Papio* northern clades			2.09 (1.54–2.71)
P. ursinus North + *P. cynocephalus* South + *P. kindae* v northern clade	1.99 (1.72–2.27)	1.76 (1.49–2.03)	
P. ursinus North + *P. cynocephalus* South − *P. kindae*	1.45 (1.19–1.72)	1.31 (1.06–1.56)	1.49 (1.03–1.98)
P. ursinus North − *P. cynocephalus* South	0.74 (0.55–0.94)	0.68 (0.51–0.87)	0.94 (0.58–1.30)
northwestern − northeastern clade	1.50 (1.26–1.74)	1.34 (1.11–1.57)	1.89 (1.33–2.48)
P. anubis West2 + *P. papio* − *P. anubis* West1	1.26 (1.03–1.48)	1.12 (0.91–1.35)	1.50 (1.02–2.02)
P. anubis West2 − *P. papio*	1.19 (0.97–1.41)	1.04 (0.82–1.26)	1.36 (0.91–1.86)
P. hamadryas + *P. anubis* East − *P. cynocephalus* North	0.39 (0.29–0.49)	0.31 (0.22–0.39)	
P. hamadryas − *P. anubis* East	0.23 (0.16–0.30)	0.16 (0.10–0.23)	

Estimates based on "Brown region" and cytochrome *b* sequence information are taken from Zinner et al. (2009).

P. anubis West1 is sister taxon to *P. papio*, or an unresolved trichotomy among the three lineages) are statistically not rejected ($P > 0.05$).

Divergence age estimates obtained from both datasets are highly similar albeit estimates from the mtDNA2 dataset are generally slightly younger than from the mtDNA1 dataset (Table 2). Accordingly, the divergence of *Papio* into the seven major haplogroups started 1.96–2.21 Ma and ended 0.68–0.74 Ma (for 95% credibility intervals see Table 2). The three lineages in haplogroup G diverged 0.31–0.39 and 0.16–0.23 Ma, respectively, and the two lineages in haplogroup B separated 0.68–0.74 Ma.

DISCUSSION

We aimed to find stronger statistical support in the phylogenetic tree reconstruction of *Papio* mitochondrial DNA, in particular for the most basal splits, and to narrow down the divergence time credibility intervals by basing our study on whole mitochondrial genomes. The second aim was accomplished by using complete mitochondrial genome information, whereas the resolution of the basal splits remained ambiguous.

The *Papio* phylogeny based on whole mitochondrial genome sequences shows a highly similar tree topology as the phylogeny based on sequences of the cytochrome *b* gene and the "Brown region" (Zinner et al., 2009, 2011a; Keller et al., 2010), but with stronger support for most nodes. Para- and polyphyletic relationships for almost all baboon species were confirmed. One possible cause for the presence of para- and polyphylies are "numts." They can be excluded here, because overlapping parts of the various amplification fragments were identical, and because of the correct translation of protein-coding genes and the forming of typical secondary structures of tRNAs and rRNAs (Thalmann et al., 2004). Incomplete lineage sorting is also highly unlikely, because this process should be random in respect to geography (Avise, 2004). However, in our mitochondrial phylogeny, we found that geographic close lineages cluster together, and, hence, introgressive hybridization remains as the most probable process leading to the observed phylogenetic discordances (Funk and Omland, 2003; Burell 2008; Zinner et al., 2009, 2011b; Jolly et al., 2011).

Estimated divergence times for all nodes are in a similar range as dates from other molecular studies (Chan et al., 2010; Perelman et al., 2011; Roos et al., 2011; Liedigk et al., 2012; Steiper and Seiffert, 2012). Also among baboon lineages, estimated divergence times are generally consistent with previous work by Zinner et al. (2009), but appear slightly younger (Table 2). The respective credibility intervals have narrowed to <55%. For example, the breadth of the credibility interval around the northeastern and northwestern divergence, estimated at 1.50 Ma from dataset mtDNA1 and 1.34 Ma from dataset mtDNA2, is reduced to 95% CIs of only 0.48 and 0.46 Ma, respectively, and thus less wide than the 95% CI of 1.15 Ma around the previously estimated divergence time of 1.89 Ma (Zinner et al., 2009).

Although the tree topology in general is strongly supported by both algorithms, some relationships identified as weakly supported in earlier studies remained ambiguous (relationship among the three western clades, *P. anubis* West1, *P. anubis* West2, and *P. papio*, and the relationship between *P. ursinus* South and all other clades). The origin of the genus lies most likely in southern Africa. This is in agreement with earlier suggestions from mitochondrial phylogenies (Newman et al., 2004; Sithaldeen et al., 2009; Zinner et al., 2009; Keller et al., 2010) and fossil data (Jablonski and Frost, 2010; Williams et al., 2012). However, the chronology of the initial divergences in southern Africa is not clear, because alternative scenarios are statistically not rejected. In our reconstruction, *P. ursinus* South diverged first followed by the main south–north split, but similarly possible are an initial south–north split or a trifurcation within a relative short time period (mtDNA1: 2.21–1.99 Ma; mtDNA2: 1.96–1.76 Ma) among the lineages leading to *P. ursinus* South, the clade consisting of *P. ursinus* North, *P. cynocephalus* South and *P. kindae*, and the northern clade.

The refined divergence dates among baboon mitochondrial lineages do not contradict the earlier hypothesis that the timing of divergence events among *Papio* lineages can be placed in a wider context related to changes in the African paleoclimate during the Pleistocene with recurrent expansions and retreats of the savanna biome as suitable habitat for baboons (Hamilton and Taylor, 1991; deMenocal, 1995, 2004; Maley, 1996; Zinner et al., 2009, 2011a, Bettridge and Dunbar, 2012). Because of periodical climate changes and the isolation and reconnection of savanna habitats, populations of baboons changed in size and spatial distribution, perhaps cyclically. Gene flow between populations occurred at differing degrees and at different times, leading to the phylogeographic pattern of baboons observed today (Zinner et al. 2009, 2011a). Although key aspects of African faunal evolution in relation to climate change remain poorly understood (Bobé et al., 2002), previous studies have already pointed out the role of glacial and interglacial changes for refugial differentiation and migration routes for the African continent (Arctander et al., 1999; Nersting and Arctander, 2001; Nyakaana et al., 2002; Lorenzen et al., 2010, 2012).

We are aware of the fact that with only mitochondrial DNA information at hand and the indications of excessive introgression, we cannot say much about the taxonomic level of the various baboon forms. However, a classification of baboon taxa as subspecies instead of species or as members of a superspecies would not change the general problem of finding closely related mitochondrial DNA in different taxa or largely different mitochondrial DNA in the same taxon. We would just shift the problem to another taxonomic level.

In conclusion, the general tree topology including paraphyletic relationships of most baboon species (Newman et al., 2004; Zinner et al., 2009; Keller et al., 2010) was confirmed by the use of whole mitochondrial sequence information and divergence times among baboon mitochondrial lineages became more reliable. This might have consequences for the use of *Papio* baboons as an analogous phylogeographic model for intra-African dispersal of hominins during the Pleistocene (Lahr and Foley, 1998). However, to fully elucidate the putative complex evolutionary history of baboon taxa and to confirm hybridization events among them, large-scale nuclear sequence data are needed.

ACKNOWLEDGMENTS

The authors thank Christina Keller, Dawit Berhane, Britta Kunz, Umaru Buba, and Julius W. Nyahongo for helping collecting samples; Christiane Schwarz for her help in the laboratory, and Tanja Haus and Gisela Fickenscher for their critical comments on an earlier version of the paper. They also thank the University of Dodoma, TAWIRI and COSTECH (Tanzania), Cape Nature House (South Africa), ZAWA (Zambia), the Direction Parcs Nationaux (Senegal), the Ministère de l'Agriculture et des Ressources Animales (Côte d'Ivoire), Gashaka Primate Project (Nigeria) and the Ethiopian Wildlife Conservation Organization for their help during sample collection.

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Chapter 4

Mitogenomic phylogeny and phylogeography of the common long-tailed macaque (*Macaca fascicularis fascicularis*)

Rasmus Liedigk[1]*, Jakob Kolleck[1], Kai O. Böker[1,2], Erik Meijaard[3,4,5], Badrul Munir Md-Zain[6], Muhammad Abu Bakar Abdul-Latiff[6], Ahmad Ampeng[7], Maklarin Lakim[8], Pazil Abdul-Patah[9], Markus Brameier[1], Dietmar Zinner[10], Christian Roos[1,11]

[1] Primate Genetics Laboratory, German Primate Center, Leibniz Institute for Primate Research, Goettingen, Germany; [2] Junior Research Group Medical RNA Biology, Primate Genetics Laboratory, German Primate Center, Leibniz Institute for Primate Research, Goettingen, Germany; [3] Borneo Futures Project, People and Nature Consulting International, Jakarta, Indonesia; [4] School of Archaeology & Anthropology, Australian National University, Canberra, Australia; [5] School of Biological Sciences, University of Queensland, St Lucia, Australia; [6] School of Environmental and Natural Resource Sciences, Faculty of Science and Technology, Universiti Kebangsaan Malaysia, Bangi, Selangor, Malaysia; [7] Sarawak Forest Department, Kuching, Sarawak, Malaysia; [8] Sabah Parks, Research and Education Division, Kota Kinabalu, Sabah, Malaysia;
[9] Department of Wildlife and National Parks, Kuala Lumpur, Malaysia;
[10] Cognitive Ethology Laboratory, German Primate Center, Leibniz Institute for Primate Research, Goettingen, Germany; [11] Gene Bank of Primates, German Primate Center, Leibniz Institute for Primate Research, Goettingen, Germany

Short title: Long-tailed macaque mitogenomics

***Correspondence to:**

Rasmus Liedigk: rliedigk@gmx.de

Published in a modified version in BMC Genomics (2015) 16:222

ABSTRACT

Long-tailed macaques (*Macaca fascicularis*) are an important model species in biomedical research, and reliable knowledge about their evolutionary history is essential for biomedical inferences. Ten subspecies have been recognized, of which most are restricted to small islands of Southeast Asia. In contrast, the common long-tailed macaque (*M. f. fascicularis*) is distributed over large parts of the Southeast Asian mainland and the Sundaland region. To shed more light on the phylogeny and phylogeography of *M. f. fascicularis*, we sequenced complete mitochondrial (mtDNA) genomes of 40 individuals from all over the taxon's range, either by classic PCR-amplification and Sanger sequencing or by DNA-capture and high-throughput sequencing. Within *M. f. fascicularis*, an initial split occurred 1.70 million years ago (Ma), separating haplotypes from mainland Southeast Asia, the Malay Peninsula and North Sumatra (Clade A), and haplotypes from Bangka, Java, Timor, Borneo, and the Philippines (Clade B). In Clade A, the three geographical populations appear as paraphyletic groups, while in Clade B local populations formed monophyletic clades with the exception of the Philippine individual which is nested within the Borneo clade. Further, in Clade B the branching pattern among main clades/lineages remained largely unresolved, most likely due to their rapid diversification 0.93-0.84 Ma. The application of complete mtDNA genomes yielded new insights into the evolutionary history of *M. f. fascicularis* by providing a more robust phylogeny and more reliable divergence age estimations which we used to discuss the phylogeography of the subspecies.

Key words

Southeast Asia, Sundaland, evolution, DNA, Sanger sequencing, high-throughput sequencing

INTRODUCTION

Fossils indicate that macaques (genus *Macaca*) arose in Northeast Africa at around 7 million years ago (Ma) [Delson, 1975; Delson, 1980]. During their expansion into Asia in the Late Miocene, the genus diversified into various species groups and species that are defined by morphological, behavioral and molecular characters, and by their geographic distribution [Fooden, 1976; Delson, 1980; Groves, 2001; Tosi et al., 2003; Ziegler et al., 2007; Riley, 2010; Anandam et al., 2013; Zinner et al., 2013a]. Macaques represent one of the most successful extant primate radiations. They are found in over 20 Asian countries and in parts of northwestern Africa, and they represent the only cercopithecine genus in Asia. The colonized geographic range, from continents to islands, is unique among non-human primates [Abegg & Thierry, 2002] and makes the genus *Macaca* an excellent example of adaptive radiation among primates [Riley, 2010].

Taxonomic affiliations of the various macaque species have been matter of debate for several decades [Fooden, 1976; Delson, 1980; Fooden, 1980; Groves, 2001; Tosi et al., 2000; Abegg & Thierry, 2002; Tosi et al., 2002; Tosi et al., 2003; Ziegler et al., 2007]. According to current classifications the genus *Macaca* comprises 22 species, which are divided into seven species groups [Zinner et al., 2013a; Roos et al., 2014; Liedigk et al., in press], among them three monotypic species groups, (1) the *M. sylvanus* group, (2) the *M. arctoides* group and (3) the *M. fascicularis* group, and four polytypic groups, (4) the Sulawesi macaques group with six species, (5) the *M. mulatta* group with three species, (6) the *M. sinica* group with five species and (7) the *M. silenus* group with five species. *M. arctoides* is most likely of hybrid origin since it either clusters with the *M. sinica* group or the *M. mulatta* group depending on the investigated genetic marker, anatomical character or behavioral trait [Fooden, 1967; Fooden, 1976; Delson, 1980; Fooden, 1980; Deinard & Smith, 2001; Tosi et al., 2003; Li et al. 2009; Perelman et al., 2011; Liedigk et al., in press]. The species composition of the *M. fascicularis* group has changed over time. Fooden [1976] and Delson [1980] included four species (*M. mulatta*, *M. cyclopis*, *M. fuscata*, *M. fascicularis*), but Groves [2001] separated *M. mulatta*,

M. cyclopis and *M. fuscata* in their own species group, the *M. mulatta* group, but integrated *M. arctoides* in the *M. fascicularis* group. Zinner et al. [2013a] likewise recognized the members of the *M. mulatta* group as distinct species group and additionally excluded *M. arctoides* proposing a monotypic *M. fascicularis* group.

The long-tailed macaque (*M. fascicularis*), as the only species of the *M. fascicularis* group [*sensu* Zinner et al., 2013a], has certainly the most discontinuous and beside rhesus macaques the largest distribution of all macaque species. Its range covers the southern part of the Southeast Asian mainland (Bangladesh, Myanmar, Thailand, Laos, Vietnam, Cambodia, peninsular Malaysia) as well as most of Sundaland (the islands of Borneo, Sumatra and Java, and adjacent islands) and beyond (central Indonesia, Philippines) (Fig. 1). On the basis of differences in pelage coloration and tail length ten subspecies are recognized [Fooden, 1980; Fooden, 1995; Fooden, 1997; Groves, 2001; Anandam et al., 2013; Zinner et al., 2013a; Roos et al., 2014; Roos & Zinner et al., in press]. Three of them (*M. f. aureus*, *M. f. fascicularis*, *M. f. philippinensis*) have relatively large distributions, while all others (*M. f. atriceps*, *M. f. condorensis*, *M. f. fuscus*, *M. f. karimondjawae*, *M. f. lasiae*, *M. f. tua*, *M. f. umbrosus*) are restricted to small islands (Fig. 1). However, no genetic data is available yet to support this classification. So far, genetic studies have included only samples from *M. f. fascicularis* and *M. f. philippinensis*. Given the large and discontinuous range of *M. f. fascicularis*, it is not surprising that (genetic) variation within this subspecies is high [Harihara et al., 1988; Tosi et al., 2003; Smith et al.; 2007; Tosi & Coke, 2007; Blancher et al., 2008; Kanthaswamy et al., 2008; Shiina et al., 2010; Berry et al., 2012; Kanthaswamy et al., 2013; Abdul-Latiff et al., 2014a; Abdul-Latiff et al., 2014b; Smith et al., 2014]. In fact, there is a deep genetic differentiation between *M. f. fascicularis* from the Asian mainland and Sundaland [Harihara et al., 1988; Tosi et al., 2003; Smith et al., 2007; Tosi & Coke, 2007; Blancher et al., 2008; Kanthaswamy et al., 2008; Berry et al., 2012; Kanthaswamy et al., 2013; Abdul-Latiff et al., 2014a; Abdul-Latiff et al., 2014b; Smith et al., 2014] and on Sumatra both Y-chromosomal lineages are found [Tosi & Coke, 2007].

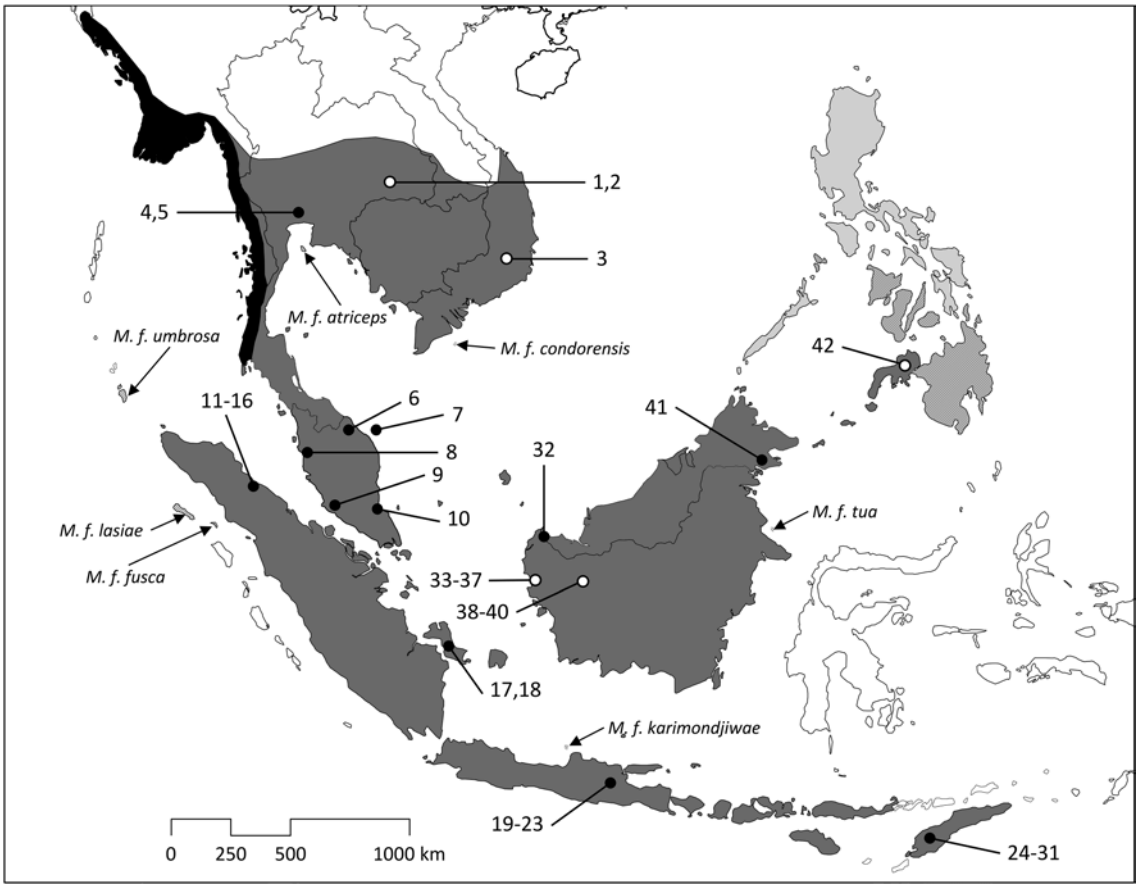

Fig. 1. Geographical distribution of long-tailed macaques (*Macaca fascicularis*) and sample collection sites. Species and subspecies distributions are depicted according to Fooden [1995] and adapted from Roos & Zinner [in press]. The distribution of *M. f. aureus*, *M. f. fascicularis* and *M. f. philippinensis* is indicated by black, dark grey and light grey regions, respectively; the hatched region indicates the transition zone between latter two subspecies. Subspecies on small islands are named in the map. Open and filled circles indicate approximate and exact geographical origin of studied *M. f. fascicularis* individuals. ID numbers correspond to those in Fig. 2 and Supporting Information Table SI.

Besides the genetic variation among local long-tailed macaque populations, genetic evidence suggests that *M. fascicularis* on the Asian mainland was introgressed by rhesus macaques [Tosi et al., 2002; Tosi et al., 2003; Bonhomme et al., 2009; Stevison & Kohn, 2009; Rovie-Ryan et al., 2013; Satkoski Trask et al., 2013a], and recent genome data indicate that around 30% of the Asian mainland *M. fascicularis* genome is of rhesus macaque (*M. mulatta*) origin [Yan et al., 2011]. This ancient hybridization (gene flow) most likely occurred unidirectional, from rhesus into long-tailed macaques and not vice versa [Tosi et al., 2002; Bonhomme et al., 2009; Stevison & Kohn, 2009; Yan et al., 2011; Rovie-Ryan et al., 2013]. Even today, hybridization between

both species occurs in a wide hybrid zone running from Vietnam, through Laos, Thailand, and probably into Myanmar [Fooden, 1997; Hamada et al., 2005]. Since the long-tailed macaque is an important model organism in biomedical research, reliable knowledge about their evolutionary history and genetic composition is essential for biomedical inferences (for an overview see [Haus et al., in press]).

The geographic origin of *M. fascicularis* and dispersal scenarios that led to its current distribution are still a matter of debate. Delson [1980] suggested that macaques entered Sundaland, probably in the Pliocene, during periods of low sea level and ancestral *M. fascicularis* became isolated there when rising sea levels and geological activity fragmented Sundaland. During the Pleistocene, *M. fascicularis* extended its range again [Delson, 1980; Fooden, 2006]. This largely corresponds to the observed higher level of nucleotide diversity found in long-tailed macaque populations from Sundaland compared to the populations from the Asian mainland and Malay Peninsula [Smith et al., 2007; Berry et al., 2012; Kanthaswamy et al., 2013; Rovie-Ryan et al., 2013; Smith et al., 2014]. This scenario is also in agreement with the fact that the earliest fossils of *M. fascicularis*, or at least those of a close relative, were found on Java [Delson, 1980; Aimi & Aziz, 1985; Fooden, 2006]. Currently, the species is also found on islands that were never connected to the Asian mainland or Sundaland, including islands east of the Wallace line (e.g., Lombok, Sumbawa, Flores, Timor) and the Philippines. Accordingly, it was assumed that humans introduced *M. fascicularis* to the islands east of the Wallace line ca. 4,000 year ago [Fooden, 2006], while the Philippines were most likely naturally colonized during two independent migration events [Smith et al., 2014]. The species' survival in other areas where it has been introduced by humans (e.g., Hong Kong, Taiwan, Papua New Guinea, New Britain, and various Pacific islands) indicates its considerable ecological plasticity. Long-tailed macaques are highly adaptable to riverine and coastal environments such as mangrove and gallery forests [Fittinghoff & Lindburg, 1980; Wheatley, 1980; Harcourt & Meijaard, 2013]. *M. fascicularis* primarily feeds on fruits and seeds [Yeager, 1996], but as indicated by one of its common names, crab-eating macaque, it also includes

crabs, shrimps, clams and fishes in its diet [Stewart et al., 2008], and is able to swim and even to dive [Son, 2003; Gumert & Malaivijitnond, 2012]. Hence, it is likely that long-tailed macaques were able to cross short distances between islands by swimming.

The Southeast Asian mainland and the Sundaland region experienced dramatic geographical and environmental changes that repeatedly occurred during the last two million years [Voris, 2000; Meijaard, 2003; Bird et al., 2005; Woodruff, 2010]. A recent biogeographic review for the region [de Bruyn et al., in press] identified three predominant sea-level scenarios for the middle to late Pleistocene in this region. The most frequently recurring scenario (±55% of last million years) is of periods with sea-levels 40-50m below current levels, leaving around half of the current Sunda Shelf emergent, and evergreen rainforests extended across much of the region. The second most frequent scenario (±37% of last million years) is of periods with very low sea levels (> 100m below current levels), such as during the Last Glacial Maximum (LGM). Seasonal vegetation was very widespread, though may not have formed a continuous north to south corridor for every glacial maximum, and the exposed and mostly vegetation free sandy soils of the shelf may have acted as a significant barrier to dispersal [Slik et al., 2011]. The degree of climatic seasonality may have varied between glacial maxima, suggested by the occurrence of certain mammalian fossils in Java. For example, faunas from the penultimate and older glacials included many large mammals, such as *Rhinoceros unicornis*, *Stegodon trigonocephalus*, *Hippopotamus sivalensis*, and *Hyaena brevirostris* requiring open woodland [Louys & Meijaard, 2010; van den Bergh et al., 2011]. The third climatic scenario is represented by the present day situation, with high sea levels and evergreen rainforests extending from the Isthmus of Kra to West Java, including Borneo. This situation, however, prevailed for just 8% of the last million years, emphasizing the ‘refugial’ nature of present day Southeast Asian rainforests and fauna [Cannon et al., 2009]. These glacial events are thought to have triggered repeated biotic range expansions between long-tailed macaques in Sumatra, the Thai-Malay Peninsula, Borneo and Java. The respective species would likely have dispersed between these areas through coastal

forests and along rivers at times of low sea-level, swimming across rivers or rafting on floating vegetation. Times of high sea-levels would have resulted in vicariance.

The objective of this study is to shed more light on the phylogeny and phylogeography of *M. f. fascicularis*, the most widespread subspecies of the long-tailed macaques, occurring on the Southeast Asian mainland and Sundaland islands, including parts of the Philippines and east as far as Timor. Therefore, we generated complete mitochondrial (mtDNA) genomes from 40 long-tailed macaque individuals either by traditional polymerase chain reaction (PCR) amplification followed by Sanger sequencing or by DNA-capture and high-throughput sequencing. We expect that the analysis of complete mtDNA genomes provide a better resolution of phylogenetic relationships among lineages than only single mtDNA fragments.

METHODS

Sample collection

We collected samples from 41 long-tailed macaque individuals originating from 16 sites throughout the species' range in Southeast Asia and Sundaland, and from the introduced population on Mauritius (Fig. 1, Supporting Information Table SI). Thirty-one of our samples (sample IDs: 4, 5, 11-31, 33-40) derived from museum specimens housed in the Bavarian State Collection of Zoology (ZSM) in Munich, Germany. Respective specimens were collected between 1904 and 1949. Dried muscle tissue attached to the skeleton was taken with sterilized scalpels and tweezers, and gloves and masks were worn during sample collection to avoid contamination. Museum samples were stored dry in tubes or plastic envelopes. Additionally, we included seven fresh fecal samples, stored in 90% ethanol, which were collected during field surveys (IDs: 6-10, 32, 41). We further obtained high-quality DNA extracted from blood samples from two individuals from Covance Inc. (Muenster, Germany) and one individual from the German Primate Center (DPZ, Goettingen, Germany) which originated from Vietnam (ID: 3; the mtDNA genome of this specimen is already published

[Liedigk et al., in press], but was herein used as bait for DNA capture) and the Philippines (ID: 42), and Mauritius (ID: 43), respectively. For all samples, we tried to obtain information about the exact geographic provenance, but this was not always possible. While for all fecal samples, GPS coordinates were recorded, information about the exact origin of the samples from Vietnam, the Philippines and Mauritius is not available. Likewise, we were not able to identify the exact provenance of five Bornean samples (IDs: 33-37, derived from "west coast Borneo"), while for all other museum samples the exact origin could be determined. Thus, 39 samples can be geographically clearly assigned to *M. f. fascicularis* (IDs: 3-41). The individual from Mauritius (ID: 43) most like refers also to *M. f. fascicularis* because it is believed that this introduced population originated from Sumatra [Tosi & Coke, 2007; Satkoski Trask et al., 2013b], while the individual from the Philippines (ID: 42) could be either *M. f. philippinensis* or *M. f. fascicularis* (due to its haplotype it is most likely *M. f. fascicularis*). For detailed sample information see Supporting Information Table SI.

All research in this project complied with protocols approved by DPZ in Germany and the Department of Wildlife and National Parks in Malaysia, 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.

DNA extraction

For the extraction of total genomic DNA we used two different methods. First, we applied a kit-based method using the First-DNA All Tissue kit (Gen-Ial). All fecal and five of the museum samples (IDs: 11, 14, 20, 31, 38) were extracted with this method following respective protocols provided by the company. To avoid and check for cross-sample contamination, all working steps were carried out in separate laboratories and under Captair Bio PCR cabinets (Erlab), gloves and masks were permanently worn, and negative extraction controls were routinely conducted. Further, samples were treated one by one,

and workbenches were decontaminated with UV light before and after every extraction. After extraction, DNA concentration was measured on a NanoDrop ND-1000 spectrophotometer and samples were stored at -20°C until further processing. Secondly, 28 museum samples (IDs: 4, 5, 12, 13, 15-31, 33-37, 39, 40) were extracted in a special ancient DNA laboratory applying a protocol for nondestructive DNA extraction [Rohland et al., 2004, 2010] with slight modifications [Haus et al., 2013]. All working steps were carried out in Thermo Scientific Safe 2020 biological safety cabinets. For each step (sample preparation, DNA extraction) different cabinets were used, and before and after each sample, cabinets were cleaned with DNA decontamination solution and treated with UV light for at least 30 min. Concentration of extracted DNAs was measured on a Qubit 2.0 fluorometer and DNA samples were frozen at -20°C until further processing. For comparative reasons, two museum samples (IDs: 20, 31) were extracted with both methods.

DNA amplification and Sanger sequencing

We generated complete mtDNA genomes from the high-quality samples from the Philippines and Mauritius as well as from three of the fecal samples (IDs: 7, 32, 41) and five of the museum samples (IDs: 11, 14, 20, 31, 38) by traditional PCR amplification followed by Sanger sequencing. All working steps (PCR setup, gel electrophoresis, PCR product purification, sequencing) were conducted in separate laboratories and under Captair Bio PCR cabinets to prevent cross-sample contamination. Further, negative PCR controls (without template DNA) were routinely conducted. To minimize the risk of amplifying nuclear mitochondrial-like sequences (numts) for the two high-quality DNA samples, we produced two overlapping long-range PCR products (8 kb and 10 kb) followed by nested PCRs with product sizes of 1.0-1.2 kb applying methods described in detail elsewhere [Liedigk et al., in press]. Since DNA extracted from fecal and museum samples is usually degraded, the complete mtDNA genome from these samples was directly amplified via 21 100-300 bp overlapping fragments and not first via two long-range PCRs. For the amplification, we used the same primers as employed above for the nested

PCRs. PCR conditions were the same as for the nested PCRs above, but sometimes the number of cycles was increased to 60. As template, we added 10-50 ng DNA to the reaction. PCR performance and product sizes were checked on 1% agarose gels, and after purification, PCR products were sequenced on an ABI 3130*xL* sequencer using the BigDye Terminator Cycle Sequencing kit (Applied Biosystems) and both amplification primers. Information on primers and PCR conditions is available upon request. Sequences were checked with 4Peaks 1.7.1 (mekentosj.com) and mtDNA genomes were assembled with SeaView 4.4.0 [Gouy et al., 2010]. Annotation was performed with DOGMA [Wyman et al., 2004] and manually verified.

DNA-capture and high-throughput sequencing

Complete mtDNA genomes from 28 museum (IDs: 4, 5, 12, 13, 15-31, 33-37, 39, 40) and four fecal samples (IDs: 6, 8-10) were generated using a DNA-capture approach followed by high-throughput sequencing according to Maricic et al. [2010] with slight modifications (see below) to adapt the workflow to the Ion PGM sequencing system (Ion Torrent). To prevent contamination, all working steps were carried out in dedicated ancient DNA and/or special high-throughput sequencing laboratories, and various negative controls were applied. After DNA extraction and concentration measurement, barcoded sequencing libraries were established using the Ion Plus Fragment Library kit and the Ion Xpress Barcode Adapters. Adapter ligation and the subsequent amplification of the samples were performed according to the protocol for Ion Xpress Plus gDNA Fragment Library Preparation. Afterwards, we pooled the adapter-ligated and amplified libraries in equal concentrations to a total of 2 µg. As bait we used mtDNA genomes of each one long-tailed macaque individual from Vietnam (ID: 3) and Mauritius (ID: 43). The respective complete mtDNA genomes were amplified via two overlapping PCR products (see above). Afterwards, we sheared the PCR products to an average of ca. 1,000 bp fragments with a Bioruptor Pico. We diluted 1.5 µg of PCR product to a volume of 150 µl, split the sample into three (50 µl each) and sonicated each six times with 10 seconds “ON” and 90 seconds “OFF”. One µl of the sheared PCR

product was size-checked on the Agilent 2100 Bioanalyzer with the high sensitivity DNA kit. Fragments were subsequently end-repaired, biotinylated by ligating the Bio-T/B adapter [Maricic et al., 2010] and immobilized on streptavidin-coated beads. Bait and the pooled single-stranded libraries were combined and four phosphorylated blocking oligos (BO1.P1.F: CCACTACGCCTCCGCTTTCCTCTCTATGGGCAGTCGGTGAT-phosphate, BO2.P1.R: ATCACCGACTGCCCATAGAGAGGAAAGCGGAGGCGTAGTGG-phosphate, BO3.A.F: CCATCTCATCCCTGCGTGTCTCCGACTCAG-phosphate, BO4.A.R: CTGAGTCGGAGACACGCAGGGATGAGATGG-phosphate) were added. After 48 h of hybridization at 65°C, library molecules that did not hybridize were washed out and the enriched library pool was eluted. Subsequently, the concentration of the enriched library pool was measured by qPCR (Ion Library Quantitation Kit) and sequenced on the Ion PGM sequencer using a 316v2 or 318v2 chip and the Ion PGM Sequencing 400 Kit protocol. The raw sequencing reads were quality-filtered, and adapters and barcodes were trimmed with the PGM Torrent Suite Software 4.2. The extracted reads were initially assembled by running the Newbler program (GS Reference Mapper) of the 454 Sequencing System Software 2.5 from command line with standard parameters. The mtDNA genome of the Vietnamese *M. fascicularis* individual (ID: 3) was used as reference. Batch processing was done by custom Perl scripts. The resulting contigs, typically ranging from 1 to 4 sequences per mtDNA genome, were manually assembled into genomes with SeaView and annotated with DOGMA. All gaps between contigs could be closed by combining the results from multiple sequencing runs.

Statistical analyses

For phylogenetic reconstructions, we expanded our dataset with additional mtDNA genome sequences from macaque and non-macaque taxa. The dataset comprised 60 mtDNA genomes including 43 *M. fascicularis* individuals, at least one representative of the other six macaque species groups (2 *M. sylvanus*, 1 *M. arctoides*, 3 *M. mulatta*, 2 *M. thibetana*, 1 *M. tonkeana*, 1 *M. silenus*) and various outgroup taxa (1 *Theropithecus gelada*, 1 *Papio hamadryas,* 1

Chlorocebus pygerythrus, 1 *Colobus guereza*, 1 *Pongo abelii,* 1 *Pan troglodytes,* 1 *Homo sapiens*). For detailed sample information and Genbank accession numbers see Supporting Information Table SI.

Sequences were aligned with Muscle 3.7 [Edgar, 2004] as implemented in SeaView and manually corrected. Indels and poorly align positions were removed with Gblocks 0.91b [Castresana, 2000] using standard settings. Identical sequences were subsequently excluded (IDs: 21=23, 27=28=31, 33=34), resulting in a final dataset of 56 unique mtDNA genome haplotypes. For Maximum Likelihood (ML) and Bayesian tree reconstructions, we applied the programs RAxML 0.93 [Stamatakis, 2006] and MrBayes 3.1.2 [Huelsenbeck et al., 2001; Ronquist & Huelsenbeck 2003], respectively. ML calculations in RAxML were run with the CAT-GTR model and 1,000 bootstrapping replications. For Bayesian tree reconstructions in MrBayes, we conducted four Markov Chain Monte Carlo (MCMC) runs with a default temperature of 0.2 and the TrN+I+G model as selected as best-fit model in jModeltest 2.1 [Posada, 2009] under the Bayesian information criterion (BIC) and the Decision Theory Performance-based Selection (DT). All repetitions were run for 1 million generations with tree and parameter sampling setting in every 100 generations. The first 25% of samples were discarded as burn-in, resulting in 75,001 trees per run. The adequacy of the burn-in and convergence of all parameters was assessed via the uncorrected potential scale reduction factor (PSRF) [Gelman & Rubin, 1992] as calculated by MrBayes and by visual inspection of the trace of the parameters across generations using TRACER 1.5 [Rambaut & Drummond, 2007]. To check whether posterior clade probabilities were also converging, AWTY [Nylander et al., 2008] was applied. Posterior probabilities for each split and a phylogram with mean branch lengths were calculated from the posterior density of trees.

Divergence ages from the dataset were estimated with BEAST 1.6.1 [Drummond & Rambaut, 2007] applying a Bayesian MCMC method with a relaxed molecular clock approach [Drummond et al., 2006]. A relaxed lognormal model of lineage variation and a Birth-Death Process prior for branching rates was assumed. The following five fossil-based calibration points were used with

a normal distribution prior for respective nodes: (1) the *Homo* – *Pan* split 6.5 Ma with a 95% credibility interval (CI) of 0.5 Ma [Vignaud et al., 2002; Brunet et al., 2005; Lebatard et al., 2008], (2) the split between *Pongo* and the *Homo-Pan* lineage at 14 Ma (95% CI: 1.0 Ma) [Kelly, 2002], (3) the divergence of *Theropithecus* and *Papio* 5 Ma (95% CI: 1.5 Ma) [Leakey, 1993; Delson, 2000], (4) the *M. sylvanus* – *M. mulatta* split at 5.5 Ma (95% CI: 1.0 Ma) [Delson, 2000; Alba et al., 2014] and (5) the divergence of hominids and cercopithecids at 27.5 Ma (95% CI: 3.5) [Zalmout et al., 2010; Pozzi et al., 2011]. Four replicates were run in BEAST for 25 million generations with tree and parameter sampling occurring every 100 generations. TRACER was used to assess the adequacy of a 10% burn-in and the convergence of all parameters via visual inspection of the trace of the parameter across generations. Sampling distributions were combined (25% burn-in) using the software LogCombiner 1.6.1. A consensus chronogram with node height distribution was generated and visualized with FigTree 1.3.1 [Rambaut, 2008].

RESULTS

We generated 42 complete mtDNA genome sequences from 40 *M. fascicularis* individuals, either by classic PCR followed by Sanger sequencing (10 individuals) or by DNA-capture and high-throughput sequencing (32 individuals). For two museum samples (IDs: 20, 31) both methods were applied which yielded identical sequences. For mtDNA genomes that were captured and sequenced on the Ion PGM sequencing platform we obtained an average of 97,583 (12,599-230,683) trimmed reads with an average read length of 96 bp, resulting in an average 285-fold coverage. Sequences in the overlapping parts were identical and all protein-coding genes were correctly translated without any premature stop codons, indicating that no numts are present in our dataset. All newly generated mtDNA genomes had a length of 16,561 to 16,567 bp, and consisted of 22 transfer RNA genes, 2 ribosomal RNA genes, 13 protein coding genes and the control region.

The original alignment for phylogenetic analysis with 60 primate sequences had a length of 16,874 bp, but was reduced to 15,868 bp after indels and poorly aligned positions were removed. Some individuals shared the same haplotype (IDs: 21=23, 27=28=31, 33=34). These were excluded resulting in a final alignment of 56 unique primate mtDNA genome haplotypes. Phylogenetic trees as obtained from ML and Bayesian analyses are mainly identical and most nodes are strongly supported (ML bootstrap values: >95%, Bayesian posterior probabilities: 1.0) (Fig. 2, Supporting Information Fig. SI). According to estimated divergence ages, Hominidae and Cercopithecidae separated 28.60 Ma (for estimates and their 95% CIs see Supporting Information Table SII). Among hominids, *Pongo* diverged from the *Homo* + *Pan* clade 13.82 Ma, while the latter split 6.32 Ma. Among cercopithecids, *Colobus* diverged first, 19.89 Ma, and *Chlorocebus* separated from papionins 12.81 Ma. In the Papionini clade, *Theropithecus* + *Papio* diverged from the macaques 10.90 Ma, while the former two genera split 4.77 Ma. Within macaques, *M. sylvanus* (from northern Africa) branched off first, 6.10 Ma. The remaining, solely Asian macaque species, diverged into two clades 5.49 Ma, one comprising *M. silenus* and *M. tonkeana*, and the other *M. thibetana*, *M. arctoides*, *M. mulatta* and *M. fascicularis*. In the former clade, *M. silenus* and *M. tonkeana* separated 3.70 Ma, while in the latter clade *M. thibetana* split off first, 4.16 Ma, followed by *M. fascicularis* 3.42 Ma, before finally *M. mulatta* and *M. arctoides* diverged 3.02 Ma. Within *M. fascicularis*, an initial split occurred 1.70 Ma, separating haplotypes from mainland Southeast Asia, Peninsula Malaysia and Sumatra (Clade A), and individuals from Borneo, Java, Bangka, Timor, the Philippines and Mauritius (Clade B). In Clade A, individuals from mainland Southeast Asia, Peninsula Malaysia and Sumatra do not form reciprocally monophyletic clades. Splitting events within Clade A occurred 0.96-0.02 Ma. In Clade B, individuals from different geographic regions form monophyletic clades or represent distinct lineages. The only exception is the Borneo clade which comprises also the individual from the Philippines (ID: 42). In clade B, the branching pattern among main clades/lineages remains unresolved indicating a diversification within a short time period. In fact, this radiation occurred between 0.93 Ma and 0.84 Ma,

thus in less than 100,000 years. Individuals from Bangka (IDs: 17, 18), an island east of Sumatra (Fig. 1), form a monophyletic clade and cluster together with the Borneo/Philippines clade. Both of these clades shared a common ancestor until 0.61 Ma. The Philippine individual is nested within the Borneo clade and specifically clusters with an individual from Sabah (ID: 41); they diverged from each other 0.21 Ma.

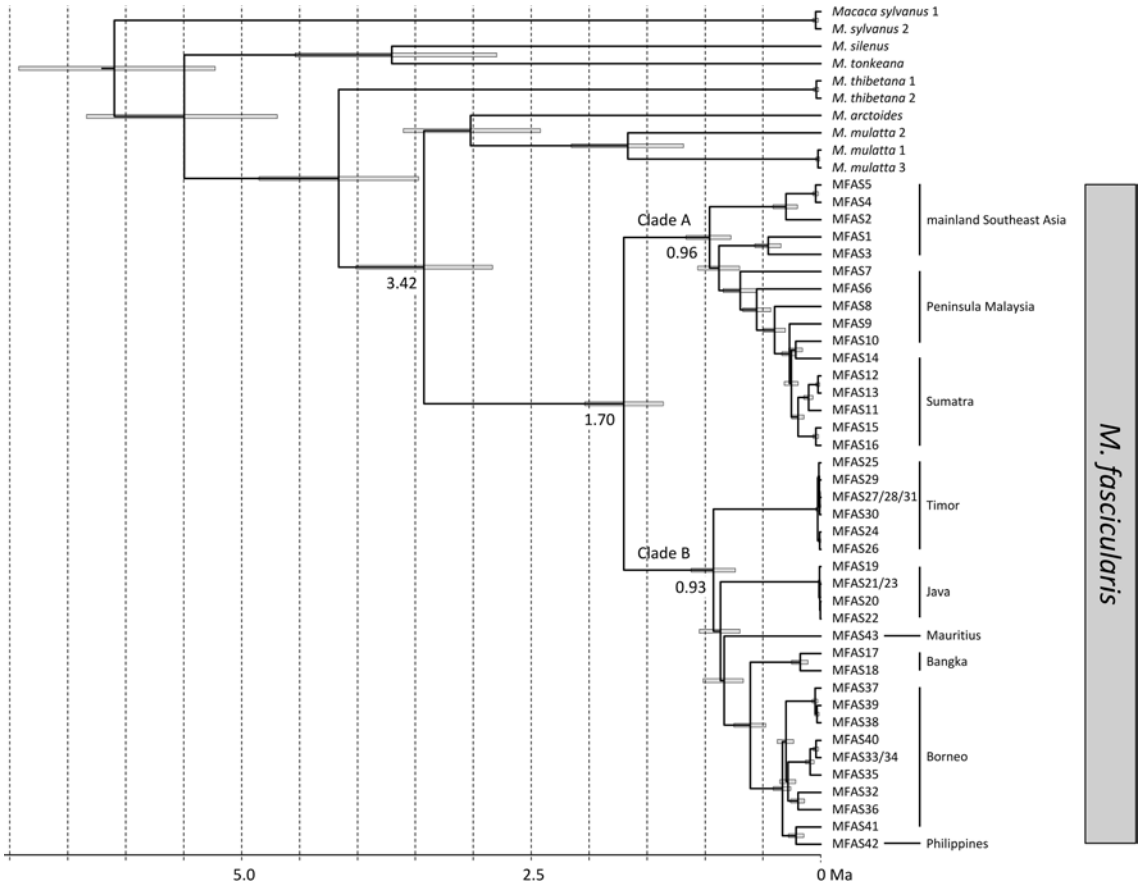

Fig. 2. Ultrametric tree showing phylogenetic relationships and divergence ages among macaques as calculated from complete mtDNA genome sequences. Grey bars indicate 95% credibility intervals of divergence times and the time scale below shows million years before present. Numbers correspond to IDs in Fig. 1 and Supporting Information Table SI. For detailed information of divergence ages and statistical node support see Supporting Information Table SII and Fig. SI, respectively.

DISCUSSION

By applying different methods, classic PCR amplification followed by Sanger sequencing and DNA-capture with subsequent high-throughput

sequencing, we successfully obtained complete mtDNA genome data from 40 *M. fascicularis* individuals. Both methods have proven to be useful to gain such data, but the DNA-capture and high-throughput sequencing approach is less cost and time intensive [Maricic et al., 2010; Gunnarsdóttir et al., 2011; Guschanski et al., 2013]. Moreover, DNA extracted from museum material and feces is normally highly degraded. Fortunately, some of our museum and fecal samples contained DNA in sufficient quality so that the complete mtDNA genome could be amplified via just 21 overlapping PCRs. Usually, due to the high grade of DNA degradation, generating complete mtDNA genomes would require a large number of PCR amplifications. In contrast, DNA capture does not need a certain DNA fragment size, because any size of DNA fragment can be captured and subsequently sequenced. However, with decreasing lengths of generated sequences, an appropriate reference sequence from a phylogenetically close taxon is required for a reliable assembly, particularly for the variable control region.

Our results concerning the phylogenetic relationships among macaque and non-macaque taxa and estimated divergence ages are largely in line with previous molecular studies [Morales & Melnick, 1998; Tosi et al., 2000; Deinard & Smith, 2001; Tosi et al., 2002; Tosi et al., 2003; Tosi & Coke, 2007; Ziegler et al., 2007; Li et al., 2009; Perelman et al., 2011; Springer et al., 2012; Finstermeier et al., 2013; Pozzi et al., 2014; Liedigk et al., in press]. For the phylogenetic relationships among *M. fascicularis* haplotypes, we gain higher statistical support for most nodes in our tree, compared to earlier mtDNA studies which mainly used only fragments of the mtDNA genome [Tosi et al., 2000; Tosi et al., 2002; Tosi et al., 2003; Smith et al., 2007; Tosi & Coke, 2007; Blancher et al., 2008; Shiina et al., 2010]. Nevertheless, some nodes in our study are still missing significant statistical support, thus leaving some phylogenetic relationships, mainly those between populations from Timor, Java, Mauritius and Bangka/Borneo/Philippines, unresolved. Such results are common when clades or lineages diverge within a short time period [Roos et al., 2011; Liedigk et al., 2012; Guschanski et al., 2013; Zinner et al., 2013b; Carbone et al., in press; Liedigk et al., in press]. In contrast to Tosi & Coke

[2007] who found Sumatran individuals to be part of the Sundaland clade, the Sumatran individuals which we used are nested within the Asian mainland clade. A possible explanation for these contradictory results is most likely the different origin of studied individuals, with the South Sumatran origin samples of Tosi & Coke [2007] clustering with Sundaland sequences and our North Sumatran samples clustering with the mainland clade. The presence of representatives of both major *M. f. fascicularis* mtDNA clades on Sumatra is further supported by the likewise presence of both Y chromosomal haplogroups on the island [Tosi & Coke, 2007].

Since mtDNA is only inherited via the maternal line and macaques live mainly in female philopatric societies [Pusey & Packer, 1987; de Ruiter & Geffen 1998], mtDNA data can be utilized to reveal insights into genetic differences among regional populations and to trace their phylogeographic history [Avise, 2004]. According to our phylogeny and estimated divergence ages, *M. f. fascicularis* initially split into an Asian mainland and a Sundaland clade 1.70 Ma, and both clades are found on Sumatra (according to our data and [Tosi & Coke, 2007]). Possible explanations are (1) Sumatra is the place of origin of *M. f. fascicularis*, (2) Sumatra is the place of origin of only Sundaland *M. f. fascicularis*, while long-tailed macaques from the mainland invaded the island later, or (3) that long-tailed macaques on Sumatra became extinct and the island was later re-colonized from the mainland and other Sundaland islands. The hypothesis that Sumatra is the place of *M. f. fascicularis* origin is supported by the observed high mtDNA diversity found on the island compared to other regions where the subspecies occurs [e.g., Smith et al., 2014]. It has been hypothesized that the northern part of Sumatra may have been separated from southern Sumatra during much of the Pleistocene due to presence of a sea strait that separated Sumatra into two islands [van Bemmelen, 1970; Meijaard, 2003; Meijaard & Groves, 2004]. This would explain the divergence of northern and southern Sumatra populations. The northern Sumatra population became reconnected to the Malay Peninsula and further to the Asian mainland via existing land bridges. The southern Sumatran population on the other hand remained separated from the Malay Peninsula and the Asian mainland, but

dispersed successively into Borneo, Java and further to the east. However, not in support of this hypothesis is the paraphyly of haplotypes from the mainland and Malay Peninsula, and the respective branching pattern among them and the Sumatra haplotypes, which suggests that the northern Sumatra population originated on the mainland. We further note that the geological evidence of a sea strait between northern and southern Sumatra remains tentative and that other factors, such as repeated volcanic eruptions in the Toba complex, could have also played a role [Nater et al., 2011; Louys, 2012; Wilting et al., 2012], even leading to the extinction of long-tailed macaques on Sumatra. To test whether Sumatra or any other island, e.g., Java [Delson, 1980; Smith et al., 2007] is the place of origin of Sundaland *M. f. fascicularis* needs further investigations and, particularly, should include data of *M. f. fascicularis* from southern Sumatra. Cercopithecid fossils from Sumatra are rather scarce compared to those from Java, and are limited to the Late Pleistocene and Holocene. They consist mainly of teeth or fragments that can hardly be assigned to particular species [Hooijer, 1962]. The paucity of clear macaque-like fossils, therefore, does not necessarily exclude Sumatra as potential origin of *M. f. fascicularis*.

As in previous studies [Tosi et al., 2003; Tosi & Coke, 2007; Smith et al., 2014], we found long-tailed macaques from the Philippines clustering within the Borneo clade. Since the Bornean individual, which is most closely related to the Philippine specimens, is from the furthest east of Borneo (Sabah, Tawau Hill Park), this branching pattern fosters the previously proposed hypothesis of a colonization of the Philippines via Borneo [Brandon-Jones, 1996; Abegg & Thierry, 2002; Smith et al., 2014]. Within the last million years, the Philippines have never been connected to the Southeast Asian mainland or Borneo via a continuous land bridge [Esselstyn et al., 2004]. Volcanic activity and uplift of oceanic crust resulted in numerous small islets between Borneo and the Philippines, which were most likely always isolated by sea channels [Heaney, 1985; Heaney, 1986; Heaney, 1991; Hall, 1998; Hall, 2002]. One possible exception is the island of Palawan which has been considered as dry land connection to Borneo during sea-level lows in the Late Pleistocene [Heaney,

1986]. Although it is not clear whether this connection has been continuous or if seawater channels interrupted it [Rohling et al., 1998; Voris, 2000], one can assume that the gap between Borneo and Palawan was relatively narrow [Esselstyn et al., 2004]. Given that long-tailed macaques are highly adaptable to riverine and coastal environments as mangrove forests and riversides [Fittinghoff & Lindburg, 1980; Wheatley, 1980], the previously proposed Philippine colonization hypothesis [Abegg & Thierry, 2002; Brandon-Jones, 1996] via Palawan and appending islets seems plausible (stepping-stone colonization). A recent study in fact suggests that there may have been at least two dispersal events from Borneo into the Philippines, first one via Palawan resulting in *M. f. philippinensis* in the north of the Philippine Archipelago, and a later one via the Sulu Archipelago that resulted in *M. f. fascicularis* in the south [Smith et al., 2014], the taxon which was included in the present study.

One noteworthy outcome from our study is the early divergence of a monophyletic Timor clade within the Sundaland clade (Fig. 2). It appears that this clade diverged some 0.93 (1.12-0.74) Ma from the other Sundaland lineages. This finding is supported by analysis of blood protein polymorphisms from samples across the Indonesian and Timor island arc which indicated that populations east of the Wallace Line (Lombok and Sumbawa) have greatly differentiated from those to the west [Kawamoto et al., 1984]. Our mtDNA-based estimate, however, significantly predates the earliest finds of macaques in Timor's archaeological record, which appear at the same time, i.e. a few thousand years ago, as the first evidence of pottery and domesticated pig in one site (Uai Bobo 1 and 2), indicating human translocations [Glover, 1986]. Similarly, on Flores, an island further west, but still east of the Wallace Line, long-tailed macaques only appear in the archaeological record around 7,000 years ago [van den Bergh et al., 2009]. It is unclear what underlies the apparent major discrepancy between the present phylogenetic analysis and the zooarchaeological record, but an introduction by humans as proposed [Fooden, 2006] seems unlikely, although the possibility remains that the detected Timor haplotypes originated from somewhere else in Sundaland, a place that was not sampled in our study.

CONCLUSIONS

Both applied laboratory methods have proven to be powerful to generate complete mtDNA genome data, with the DNA-capture and high-throughput sequencing approach as the most promising and only option to obtain such data from highly degraded DNA, in time and with relatively low costs. Nevertheless, appropriate reference sequences from phylogenetically close taxa are essential for reliable assemblies, particularly for the variable control region.

Our study provides new insights into the evolutionary and phylogeographic history of *M. f. fascicularis*, most prominent the confirmed mainland clade in North Sumatra and the clearly distinct and old Timor clade. However, to identify the origin of long-tailed macaques and their dispersal routes leading to their current distribution, to assess their full genetic diversity and to explore to which extent secondary gene flow occurred between local populations, it is fundamental to include further *M. f. fascicularis* populations from throughout their range into the studies. In these studies both, mitochondrial and a large number of nuclear loci, should be analyzed. Moreover, to fully understand the evolutionary and phylogeographic history of the species, the other subspecies of *M. fascicularis* should be incorporated in such studies as well.

ACKNOWLEDGEMENTS

We are grateful to the Bavarian State Collection of Zoology, Covance Inc. and the German Primate Center for providing valuable long-tailed macaque samples, as well as to the Universiti Kebangsaan Malaysia, Department of Wildlife and National Parks (PERHILITAN), Sarawak Forest Department, Sabah Wildlife Department and Sabak Parks for sharing fecal samples. This research was financially supported by the German Primate Center and by grants to BM Md-Zain (FRGS/1/2012/STWN10/UKM/02/3, DLP-2013-006, ERGS/1/2013/STWN10/UKM/02/1). We further thank Christiane Schwarz, Nico Westphal and Jens Gruber for support during laboratory work and statistical analysis.

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Chapter 5

General Discussion

5.1 Summary and discussion

The cercopithecine tribe Papionini represents one of the most successful primate radiations in terms of diversity and distribution. However, phylogenetic relationships within this tribe are still not satisfactorily resolved and important questions about the evolutionary history of the tribe remain unclear. The objective of my thesis was therefore to contribute to our understanding of the evolution of the Papionini by focussing on the mitogenomic relationships within the tribe. The tribe comprises 45 species of which 20 are represented in this study. Altogether, I generated complete mitochondrial genomes (Sanger sequencing) of 28 Papionini individuals. To generate mitochondrial sequence data, I used blood samples obtained from zoos and breeding facilities (chapter 2) and faecal samples which were collected for earlier studies (Keller et al., 2010; Zinner et al., 2009) (chapter 3). In addition, I gathered dried tissue samples from museum specimens (chapter 4) of which 30 were used to generate mitochondrial genome sequences via DNA-capture and high-throughput sequencing.

To investigate phylogenetic relationships on different taxonomic levels the following three studies were conducted:
(1) An investigation of the mitogenomic relationships and divergence times of all genera (except *Rungwecebus*) and *Macaca* species groups of the tribe Papionini (chapter 2). (2) An analysis of the mitogenomic relationships among the six baboon species representing ten different mitochondrial haplogroups to test whether complete mtDNA genomes reveal a better resolution of phylogenetic relationships than previous analyses using only short mtDNA sequences (chapter 3). And (3) an analysis of the intra-specific relationships and divergence times among *Macaca fascicularis* individuals using samples from throughout the species' range (chapter 4).

Results show basically well-resolved and highly supported phylogenies and divergences ages with narrowed confidence intervals. However, the results are in

some cases contradictory to respective nuclear studies and some relationships still remain ambiguous and need further investigation.

As the main findings of study 1 (chapter 2) we found three mitochondrial clades: (1) *Papio*, *Theropithecus*, *Lophocebus*; (2) *Mandrillus*, *Cercocebus*; (3) *Macaca*. Remarkably, the *Macaca* clade appears as sister clade to *Mandrillus* and *Cercocebus*, a finding that is discordant to morphological and recent nDNA studies (Gilbert, 2008; Perelman et al., 2011; Page & Goodman, 2001; Xing et al., 2005). A similar finding had been reported from other mtDNA studies (Finstermeier et al., 2013; Pozzi et al., 2014). In this context, we found that an increased taxon sampling can influence phylogenetic results, since alternative positions of the *Mandrillus* + *Cercocebus* clade were statistically rejected, which was not the case in Finstermeier et al. (2013).

Our data reveal paraphyletic relationships within the *Mandrillus* + *Cercocebus* clade and among *Papio* taxa, whereas relationships between *Theropithecus*, *Lophocebus* and *Papio* remain ambiguous. The most likely reason for the latter is that the three lineages diverged within a relatively short time period (between 5.2 and 4.7 Ma), which makes a clear resolution difficult. Contemporary gene flow between *Papio* and *Theropithecus* was reported (Dunbar & Dunbar, 1974) and might have also occurred historically and contributed to ambiguous relationships among the three lineages.

The divergence age estimation further revealed initial splits within the three major mitochondrial clades (*Papio* + *Theropithecus* + *Lophocebus*; *Mandrillus* + *Cercocebus*; *Macaca*) at the Miocene/Pliocene boundary and Papionina genera diverged at a similar time scale as *Macaca* species groups. Hence, the mitochondrial heterogeneity among macaque species groups is at least as high as among African genera. Whether this warrants a respective taxonomic reorganisation is further discussed in section 5.4.

In study 2 (chapter 3) we found seven major mt-haplogroups among ten baboon populations, indicating paraphyletic relationships of several *Papio* species. Phylogenetic relationships, especially the most basal splits, remain unresolved although support values were relatively high. This corroborates results from earlier studies which were based on partial mtDNA sequences (cytochrome b, Brown region; Zinner et al., 2009; Keller et al., 2010). Also the obtained divergence dates are consistent with previous studies (Zinner et al., 2009), but appear slightly younger,

whereas credibility intervals have narrowed. Although node support improved, the obtained mitochondrial tree topology reflects the geographic distribution of respective taxa and not monophyletic species clades. We obtained the same paraphylies and similar divergence times within *Papio* after combining the respective *Papio* sequences with the Papionini dataset (chapter 2). The paraphylies among *Papio* species might be the result of repeated ancient gene flow among different *Papio* lineages, a process that is still ongoing and reported from most contact zones, especially for olive and hamadryas baboons in Ethiopia and olive and yellow baboons in Amboseli (Alberts & Altmann, 2001; Bergmann et al., 2008; Nagel, 1973; Shotake, 1981; Tung et al. 2008; Zinner et al., 2011).

In study 3 (chapter 4), we found a continental and an insular *Macaca fascicularis* clade which separated at 1.7 Ma. This finding confirmed results of earlier genetic studies (e.g., Harihara et al., 1988; Tosi et al., 2002, 2003). Furthermore, our mitochondrial data indicate the presence of the continental lineage in North Sumatra. However, whether both haplogroups have restricted ranges or whether both are distributed all over the island remains unknown so far. Tosi and Coke (2007) studied only long-tailed macaques from South Sumatra and found in them only the insular haplogroup, whereas these animals carried both, the continental and the insular Y-chromosomal haplogroups. Our study further reveals a rapid radiation of *M. fascicularis* on the Sunda Shelf 0.93-0.84 Ma.

5.2 The mitochondrial genome and phylogenetic reconstructions

In summary one can say that complete mitochondrial genomes in general result in mainly well-supported phylogenies, due to the increased number of informative sites compared to shorter mtDNA fragments. However, our results in study 1 and 2 also show some limitations of such approaches, e.g., paraphyletic relationships among baboons and mangabeys and unresolved relationships among *Theropithecus*, *Lophocebus* and *Papio* or the lineages/clades within the *M. fascicularis* insular clade. Incomplete lineage sorting and secondary gene flow are possible explanations for paraphyletic relationships among baboon and mangabey taxa. Hybridisation has been discussed as reason for tree topology discordances in many studies (e.g., Finstermeier et al., 2013; Keller et al., 2010; Liedigk et al., 2012; Roos et al., 2011;

Tosi et al., 2002; Zinner et al., 2009, 2011). An example that illustrates discordances between gene trees of Asian colobines as inferred from mitochondrial and nuclear data is shown in Figure 5.1 (Liedigk et al., 2012). The mtDNA phylogeny suggests paraphyly of *Rhinopithecus bieti*, which is monophyletic in the nDNA phylogeny. A similar pattern is shown among *Pygathrix* taxa. Both discordances are most likely the result of secondary gene flow between respective lineages. This is just one example showing that the mitochondrial genome represents just one locus and respective phylogenies reveal only a certain, but important aspect of the evolutionary history of taxa. Often the phylogeographic histories of populations or taxa are better preserved in the geographic pattern of mtDNA than in the nuclear genomes of the respective populations (Avise 2000), at least when respective taxa live in female philopatric societies as most primates do (Pusey & Packer, 1987). Nevertheless, for a complete understanding of the evolutionary history of a species other loci (nDNA) have to be included as well. If such multi-locus nDNA phylogenies are congruent witch each other and with those inferred from mtDNA it could be an indication that the depicted gene trees actually reflect the true species phylogeny (Avise, 2004; Moore, 1995). On the other hand, if nDNA and mtDNA topologies differ, incomplete lineage sorting or secondary gene flow might have influenced the inferred topologies. However, often it remains open, which of both scenarios is responsible for respective discordances. The analyses of whole genomes might contribute to solve this issue and to figure out to which extent hybridisation influenced the evolutionary history of species. With the advent of high-throughput sequencing techniques more and more studies focus on whole genome comparisons, e.g., Gibbs et al., 2007; Higashino et al., 2012; Yan et al., 2011 (macaques), Carbone et al. in press (gibbons), Ebersberger et al., 2002; Li & Durbin, 2011; Sequencing TC & AC, 2005 (chimpanzees, humans). In the case of macaques Yan et al. (2011) found out that around 30% of the Asian mainland *M. fascicularis* genome is of *M. mulatta* origin. Other molecular studies have shown that hybridisation between the *M. fascicularis* and *M. sinica* lineage formed *M. arctoides* which has a mosaic-like genome comprising genes from both parental lineages (Li et al., 2009; Tosi et al., 2000, 2003; Zinner et al., 2011). Even the human genome has experienced introgression from at least three archaic hominin species (Neanderthals, Denisovans and unknown hominin) (Green et al., 2010; Reich et al., 2010; Prüfer et al. 2014). Single locus analyses often brought up the assumption that introgressive hybridization influenced

respective phylogenies and with whole genome analyses it is possible to determine more precisely to which extent introgression occurs, since not all parts of the genome are effected equally by hybridisation (Zinner et al., 2011). In this context genome-wide Single Nucleotide Polymorphisms (SNPs) detection is of importance, a method to assess genetic variation among populations. Since not all parts of the genome are effected equally by introgression and also not all individuals of a population carry the hybrid genetic make-up in their genome, multi-locus approaches or even the analyses of whole genome data is necessary to resolve, or at least to approximate the complete evolutionary history, hence the species tree of a certain taxon. In the case of gibbons it has recently been shown that even complete genome analysis does not necessarily lead to resolved phylogenies (Carbone et al. in press).

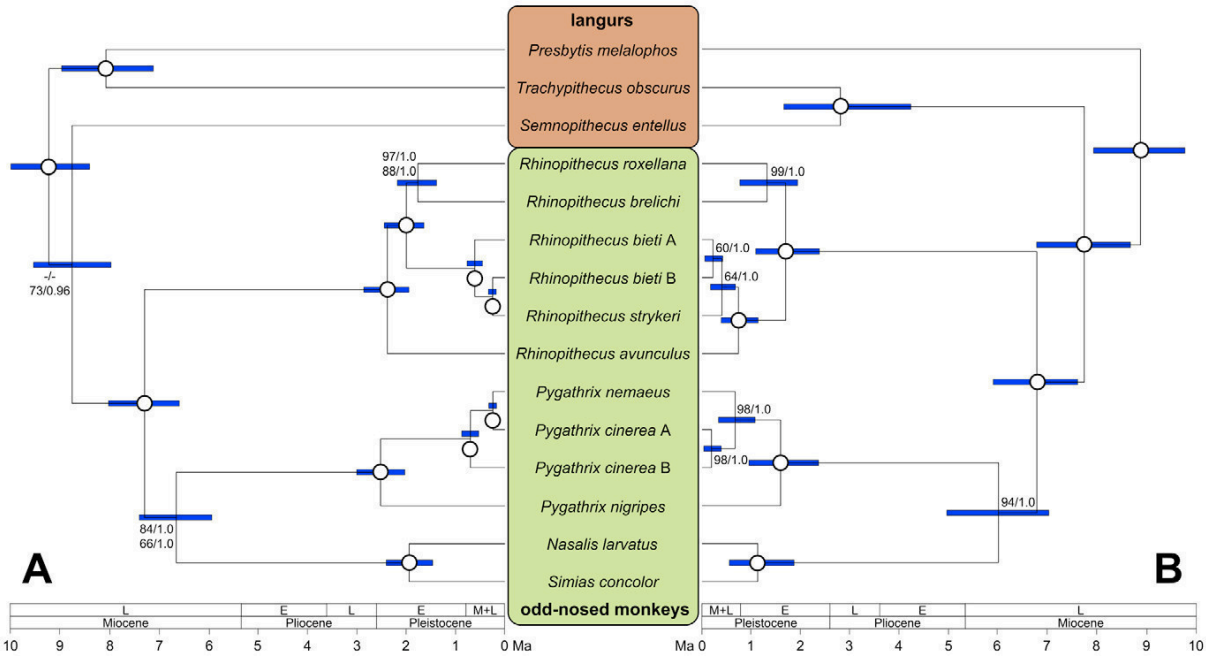

Figure 5.1: Ultrametric tree showing phylogenetic relationships among Asian colobines as obtained from mitochondrial (A) and nuclear sequence data (B). Open circles indicate ML bootstrap values of 100% and posterior probabilities of 1.0; values below are given at respective branches. Blue bars represent 95% highest posterior densities of divergence ages. In A, upper and lower numbers on branches indicate ML bootstrap values and posterior probabilities as derived from datasets mtDNA1 and mtDNA2, respectively. Abbreviations used in the bars: L = late, E = early, and M= middle. (Liedigk et al., 2012).

5.3 Fossils and divergence ages

Our estimated mitochondrial divergence ages between Papionini genera (chapter 2), between *Papio* spp. (chapter 3) and between *Macaca fascicularis* ssp. (chapter 4)

are mainly in agreement with previous studies (e.g., Finstermeier et al., 2013; Keller et al., 2010; Tosi et al., 2003; Zinner et al., 2009) and further correspond to the papionin fossil record. The earliest macaque fossils outside of Africa, dated to the Late Miocene, were found in southern Europe and were assigned to the *M. sylvanus* lineage (Alba et al., 2014; Köhler et al., 2000). Our divergence age estimation revealed that the African and the Asian macaque lineages separated around the same time (~6 Ma). This is slightly before guenons (Cercopithecini) left Africa (dated by fossils from Arabia at 6.5-8 Ma; Gilbert et al., 2014). Successive divergence events of the *Macaca* species groups are mainly in line with the dispersal scenarios proposed by Fooden (1976, 1980). The *M. silenus* lineage (represented in chapter 2 by *M. silenus* and *M. tonkeana*) split off from the remaining macaques at ~5 Ma and formed the first dispersal wave after *M. sylvanus*. Next, at around 4 Ma the *M. sinica* lineage (*M. thibetana*) split off, representing Fooden's second dispersal wave. The earliest described macaque fossil in Asia has been dated to ~4 Ma (China) (Alba et al., 2014), but was not assigned to any species. The earliest fossils associated with the *M. sinica* lineage were discovered from Early Pleistocene excavations in China.

According to the estimated divergence ages and the fossil record of *Theropithecus*, one can infer that the lineage arose in Africa and remained on the continent until the Early Pleistocene (Belmaker, 2002; Delson, 1993; Delson et al., 1993; Delson, 2000; Gibert et al., 1995; Gupta & Sahni, 1981; Leakey, 1993; Pickford, 1993; Roberts et al., 2014; Rook et al., 2004, 2013).

5.4 Phylogenetic and taxonomic implications

One of the main aims of my thesis was to investigate phylogenetic relationships within the Papionini on different taxonomic levels. Some results reveal that defining taxonomic groups is still a challenging task. Due to the mtDNA phylogeny and divergences ages (chapter 2), splits between *Macaca* species groups are as deep or even deeper as among African genera. Goodman (1998) and Groves (2001; 2004) proposed that taxonomic ranks above species level should be linked to certain time depths. For primates, Groves (2004) suggested for the genus level the Miocene/Pliocene boundary (5.3 Ma), which would correspond to the initial splits in the three Papionini clades and would be in favour of a three-genera-classification. In

turn, one could argue that macaque species groups should be elevated to genera, given that their African sister taxa are classified on the genus level. However, when considering the morphological similarities of macaques compared to the prominent morphological differences between African genera, a reorganisation of taxonomic ranks of papionins based on time depths seems to be unjustified.

The intra-generic study (chapter 3) revealed paraphyly among almost all baboon species, indicating that complete mitochondrial genomes are not sufficient to identify traditional *Papio* species as delineated by morphological characters. All six baboon species are clearly distinguishable by morphological characters, but based on mitochondrial markers, they do not form monophyletic groups. Groves (2004) proposed that the criterion of monophyly is mandatory above the species level but not for species. However, mitochondrial paraphyly is also found among genera that are morphologically clearly distinct from each other (e.g., *Mandrillus* and *Cercocebus*).

Species concepts

"A group of actually or potentially interbreeding natural populations which is reproductively isolated from other such groups." This is the well-known definition of the Biological Species Concept (BSC) (Mayr, 1940, 1942, 1963). The BSC is only one species concept among more than 20 others (Groves, 2012), however it is the most prominent one. With regard to results from many former studies the BSC has been proven inapplicable in many cases since interbreeding among different species and even among different genera is a common phenomenon (Zinner et al., 2011).

Alternatively, the Phylogenetic Species Concept (PSC) defines a species as "the smallest cluster of individual organisms within which there is a parental pattern of ancestry and descent and that is diagnosably distinct from other such clusters by a unique combination of fixed character states" (Cracraft, 1983, 1997). In contrast to the BSC, the PSC does not imply the criterion of reproductive isolation, but focuses on any kind of fixed unique characters, be it morphology, behaviour or genetics. According to the PSC, the six baboon species are clearly diagnosable by morphological characters.

5.5 Biogeographic implications

In evolutionary biology, direct observation of evolutionary processes are difficult and in most cases impossible. Therefore, comparative approaches are an important tool for inferring evolutionary history and adaptation (Clutton-Brook & Harvey, 1984; Harvey & Purvis, 1991; Harvey & Pagel, 1991). Furthermore, many questions in evolutionary biology can be studied only if one applies analogues models, e.g. if one uses model species. In order to study the phylogeography of humans, e.g. their dispersal within Africa and out of Africa, two papionin taxa, *Papio* and *Macaca*, has been suggested as informative models. The genus *Papio*, inhabiting most of sub-Saharan savannah habitats, has evolved in parallel to humans during the last 2.5 Ma in similar, if not in the same habitats as humans (Garrigan & Kingan, 2007; Jolly, 2001; Kopp et al. in press; Newman et al., 2004) and the genus *Macaca* can be used as a model for an out-of-Africa dispersal into Eurasia (Andrews et al., 1996; Fooden, 1976, 1980).

Our baboon phylogeny and respective divergence age estimations revealed an initial split into a southern and a northern clade at ~2 Ma, followed by an east-west split of the northern clade around 1.5 Ma. These divisions were most likely triggered by climatic changes and respective transformation of the environment. Similar phylogeographic patterns were also observed in other African savannah mammals, e.g., antelopes (*Alcelaphus buselaphus*, *Connochaetes taurinus*, *Domaliscus lunatus*, *Hippotragus equinus*), giraffes (*Giraffa camelopardalis*), warthogs (*Phacochoerus africanus*), lions (*Panthera leo*) (Alpers et al., 2004; Arctander et al., 1999; Flagstad et al., 2001; Hassanin et al., 2007; Muwanika et al., 2003; Barnett et al., 2006). Possibly, early savannah-inhabiting humans followed the same dispersal routes during the Pleistocene.

The human lineage dispersed out of Africa most likely during the Early Pleistocene, but the origin and causes of dispersal are not well understood (Antón et al., 2002; Fleagle et al., 2010). Although not on the same time scale, the genus *Macaca* might represent a useful analogue model to infer human dispersal scenarios (Antón et al., 2002).

5.6 Conclusion and outlook

My thesis provides a comprehensive overview of the mitochondrial diversity among papionin taxa. Although the mitogenomic phylogenies provide normally higher support values than trees derived from shorter mitochondrial sequences, the data further show that the mitochondrial genome as a phylogenetic marker does not allow phylogenetic resolution equally on all taxonomic levels and also does not warrant species delimitation in many cases. Paraphyletic relationships and discordances to nDNA studies indicate that hybridisation and/or incomplete lineage sorting affected most of papionin lineages. To detect hybridisation among the Papionini and to get an approximation to real species trees instead of single gene trees, intensive analyses of nuclear loci or even of whole genomes of the respective taxa are needed. Beside multi-locus approaches, future studies should further increase the sample size per species to reduce phylogenetic error (Nabhan & Sarkar, 2012; Pollock et al., 2002; Townsend & Leuenberger, 2011; Zwickl & Hills, 2002). Emphasis should be placed on primate groups that are understudied in terms of their phylogenetic relationships such as the mangabeys (*Cercocebus*, *Lophocebus*, *Rungwecebus*). Here, in addition to samples from the field, more specimens from museum and other collections should be included in the analyses (e.g. for guenons, Guschanski et al., 2013). These collections provide a comprehensive source of material which can be efficiently analysed with new DNA-capture methods and high-throughput sequencing techniques. Museums might also hold taxa or populations that became already extinct and which cannot be obtained from the field anymore, but which might contribute informative insights to future phylogenetic analyses. Moreover, in addition to molecular data, morphological as well as ecological and behavioural data should be taken into account to get a comprehensive picture of the evolutionary history of taxonomic groups.

Summary

The present study is meant to further illuminate the evolutionary history of the cercopithecine tribe Papionini. The Papionini, as sister lineage to the Cercopithecini, belong to the Cercopithecinae subfamily and comprise seven genera (*Macaca*, *Cercocebus*, *Mandrillus*, *Lophocebus*, *Papio*, *Theropithecus*, *Rungwecebus*) and 45 species. Six of the seven genera are today mainly restricted to Africa with the exception of *Papio* of which one species, *P. hamadryas,* is found also in Southwest Arabia. In contrast, the seventh genus, *Macaca*, is mainly an Asian taxon with only a small range in Northwest Africa (*M. sylvanus*). The fossil record indicates that the genera *Macaca* and *Theropithecus* occurred also in Europe during the Plio-Pleistocene. *Theropithecus* is today restricted to Africa but Pliocene fossils were discovered in North India.

Phylogenetic relationships among papionin taxa have been analysed applying morphological and genetic traits, but respective results were not completely concordant. Particularly, the phylogenetic relationships between *Theropithecus*, *Lophocebus* and *Papio*, and relationships within the genus *Papio* as well as among *Macaca* species groups are not satisfactorily resolved so far.

To shed more light on the evolutionary history of the Papionini I conducted three studies (chapter 2-4) to investigate inter- and intra-generic as well as intra-specific relationships. For this purpose I generated complete mitochondrial genomes to reconstruct phylogenetic trees and to estimate divergence ages. Results reveal three major clades within the Papionini (chapter 2): (1) *Papio*, *Theropithecus*, *Lophocebus*; (2) *Mandrillus*, *Cercocebus*; (3) *Macaca*, whereas the latter appears as sister clade to *Mandrillus* and *Cercocebus* and not as sister lineage to all African Papionini. This finding is in discordance to nuclear and morphological studies. The results further show that complete mitochondrial genomes are in some cases not sufficient to resolve phylogenetic relationships as for example between *Theropithecus*, *Lophocebus* and *Papio* (chapter 2). The dataset further reveals paraphyletic relationships among *Mandrillus* and *Cercocebus* (chapter 2) as well as within *Papio* (chapter 3). In the latter case, baboon mtDNA-clades cluster according to their geographic origin and not according to their taxonomy, making most baboon species paraphyletic. These branching patterns are most likely caused by secondary gene flow between parapatric baboon species. In the third study (chapter 4), in which

intra-specific relationships within the Asian long-tailed macaque (*Macaca fascicularis*) were analysed, we found a clear division into a continental and an insular clade. Both, continental and insular lineages were found on Sumatra, indicating secondary gene exchange between continental and insular populations.

In general, the results show that complete mitochondrial genome sequences result in well-resolved and highly supported phylogenies which provide basic phylogenetic information for future comparative studies. Divergence age estimations are mainly concordant with earlier studies but confidence intervals narrowed. However, it has also been shown that mitogenomic phylogenies do not reveal high resolutions when taxa diverge within short time periods. The detected paraphyletic relationships and discordances to nuclear studies are most likely the result of incomplete lineage sorting or secondary gene flow. Hence, for a complete understanding of the evolutionary history of taxa, multi-locus approaches including nuclear data are essential, since mitochondrial phylogenies represent only a single gene tree and thus only one, but important, aspect of the evolutionary history of taxa.

Zusammenfassung

Die vorliegende Arbeit soll dazu beitragen, Unstimmigkeiten in den Verwandtschaftsverhältnissen innerhalb der Papionini, einem Stamm innerhalb der Altweltaffen (Cercopithecidae), zu klären. Die Papionini, die zusammen mit den Cercopithecini die Unterfamilie der Cercopithecinae bilden, beinhalten sieben Gattungen (*Macaca*, *Cercocebus*, *Mandrillus*, *Lophocebus*, *Papio*, *Theropithecus*, *Rungwecebus*) und 45 Arten. Sechs der sieben Gattungen kommen heute hauptsächlich in Afrika vor. Eine Ausnahme ist die Gattung *Papio*, die mit einer Art (*P. hamadryas*) auch in Südwest-Arabien vorkommt. Im Gegensatz zu den sechs hauptsächlich afrikanischen Gattungen hat die siebte Gattung (*Macaca*) nur ein kleines Verbreitungsgebiet im Norden Afrikas und kommt sonst hauptsächlich in Asien vor. Fossilfunde belegen allerdings, dass während des Plio- und Pleistozäns die Gattungen *Macaca* und *Theropithecus* auch in Europa vorkamen. Von der Gattung *Theropithecus*, die heute ausschließlich in Afrika beheimatet ist, wurden zudem auch Fossilien aus dem Pliozän im Norden Indiens gefunden.

Die Verwandtschaftsbeziehungen innerhalb der Papionini wurden bisher mit Hilfe morphologischer und genetischer Merkmale untersucht, allerdings waren die Ergebnisse nicht immer übereinstimmend und es gibt immer noch viele Unklarheiten. Zum einen ist nicht eindeutig geklärt, wie die Gattungen *Papio*, *Lophocebus* und *Theropithecus* zu einander in Beziehung stehen. Zum anderen ist auch unklar, wie die einzelnen Pavianarten innerhalb der Gattung *Papio* mit einander verwandt sind. Außerdem sind auch die Verwandtschaftsverhältnisse zwischen und innerhalb der Artgruppen der Makaken nicht eindeutig geklärt.

Um mehr Klarheit in die Evolution der Papionini zu bringen, habe ich im Rahmen dieser Arbeit drei Studien durchgeführt (Kapitel 2-4). Ziel dabei war es, Verwandtschaftsbeziehungen auf unterschiedlichen taxonomischen Ebenen (zwischen und innerhalb von Gattungen, sowie innerhalb einer Art) zu untersuchen. Dazu wurden komplette mitochondriale Genome von Vertretern der Papionini sequenziert und damit Phylogenien und Aufspaltungszeiten berechnet. Die Ergebnisse meiner Arbeit zeigen unter anderem drei Hauptkladen innerhalb der Papionini (Kapitel 2): 1) *Papio*, *Theropithecus*, *Lophocebus*; 2) *Mandrillus*, *Cercocebus*; 3) *Macaca*, wobei *Macaca* in der mitochondrialen Phylogenie näher mit *Mandrillus* und *Cercocebus* verwandt zu seien scheint und nicht wie erwartet, als

Schwestergruppe der afrikanischen Papionini abgebildet wird; ein Ergebnis, das im Widerspruch zu nukleären und morphologischen Studien steht.
Meine Arbeit zeigt auch, dass komplette mitochondriale Genome in manchen Fällen nicht ausreichen, um phylogenetische Beziehungen vollständig zu rekonstruieren. So bleibt weiterhin unklar wie die Gattungen *Papio*, *Theropithecus* und *Lophocebus* zueinander stehen (Kapitel 2). Außerdem zeigen die Ergebnisse Paraphylien für *Mandrillus* und *Cercocebus* (Kapitel 2), sowie innerhalb der Paviane (Kapitel 3). Die Paviane werden dabei gemäß ihrer geographischen Verbreitung und nicht nach ihrer taxonomischen Zugehörigkeit abgebildet, wodurch die meisten Pavian-Arten paraphyletisch sind. Der Grund für diese Baumtopologie ist sehr wahrscheinlich sekundärer Genfluss zwischen parapatrisch vorkommenden Pavian-Arten. In der dritten Studie (Kapitel 4), in der innerartliche Verwandtschaftsverhältnisse innerhalb einer südostasiatischen Makaken-Artgruppe (*Macaca fascicularis*) untersucht wurden, zeigt sich eine klare Unterteilung in eine kontinentale und eine insulare Klade. Sowohl die kontinentale, als auch die insulare Linie sind auf Sumatra zu finden, was für einen sekundären genetischen Austausch zwischen beiden Populationen spricht.

Generell kann man sagen, dass komplette mitochondriale Genome robuste Phylogenien mit hoher statistischer Unterstützung ergeben, die eine gute Grundlage für künftige vergleichende Studien bilden. Die berechneten Aufspaltungszeiten stimmen weitestgehend mit vorherigen Studien überein, wobei sich die ermittelten Konfidenzintervalle verkleinert haben. Allerdings zeigt die Arbeit auch, dass Phylogenien basierend auf mitochondrialen Genomen keine hohe Auflösung erzielen wenn sich Taxa innerhalb kurzer Zeit voneinander trennten. Die hier gezeigten Paraphylien und die abweichenden Ergebnisse zu nukleären Studien wurden höchstwahrscheinlich durch sekundären genetischen Austausch hervorgerufen. Um Verwandtschaftsverhältnisse möglichst exakt rekonstruieren zu können, müssen neben der maternal-vererbten, mitochondrialen Linie noch paternal- und biparental-vererbte Merkmale in Betracht gezogen werden. Zu beachten ist in diesem Zusammenhang, dass ein bestimmter molekularer Marker immer nur eine mögliche Phylogenie von vielen wiedergibt.

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Chapter 2

Table S1. Divergence ages among catarrhine primates in Ma (95% credibility intervals) estimated with uncorrelated and auto-correlated relaxed clock models based on dataset 1.

	uncorrelated (BEAST)	uncorrelated (PhyloBayes)	autocorrelated (PhyloBayes)	
Split	mtDNA divergence ages	mtDNA divergence ages	mtDNA divergence ages	nDNA divergence ages[1]
Cercopithecoidea – Hominoidea	27.12 (23.62-30.83)	27.42 (24.23-30.76)	24.62 (24.02-26.19)	31.56 (25.66-37.88)
Pongo – (*Homo* + *Pan*)	13.86 (12.74-15.01)	13.82 (13.04-14.90)	13.75 (13.03-14.88)	16.52 (13.45-19.68)
Homo – *Pan*	6.41 (5.81-6.98)	6.45 (6.02-6.97)	6.59 (6.04-6.98)	6.60 (5.40-7.96)
Colobinae – Cercopithecinae	18.47 (13.89-24.03)	22.66 (18.21-27.29)	15.78 (13.00-18.87)	17.57 (13.88-21.52)
Papionini – *Chlorocebus* (Cercopithecini)	12.51 (9.77-15.72)	15.58 (12.03-19.69)	11.50 (9.53-13.68)	11.50 (9.18-13.85)
(*Macaca* + *Mandrillus* + *Cercocebus*) – (*Papio* + *Theropithecus* + *Lophocebus*)	10.69 (8.24-13.20)	12.68 (9.83-16.31)	10.21 (8.69-11.95)	-
Macaca – remaining Papionini	-	-	-	8.13 (6.69-9.68)
(*Mandrillus* + *Cercocebus*) – (*Papio* + *Lophocebus* + *Theropithecus*)	-	-	-	6.67 (5.37-8.07)
Macaca – (*Mandrillus* + *Cercocebus*)	9.41 (7.34-11.91)	10.82 (8.29-14.14)	9.45 (8.23-10.81)	-
Theropithcus – (*Papio* + *Lophocebus*)	5.20 (4.04-6.41)	5.24 (3.79-6.42)	6.11 (5.28-6.49)	4.06 (3.36-4.70)
Papio – *Lophocebus*	4.70 (3.59-5.92)	4.70 (3.33-6.04)	5.87 (5.06-6.35)	3.24 (2.46-4.07)
T. gelada 2 – (*T. gelada* 3 + *T. gelada* 1)	0.30 (0.16-0.50)	0.30 (0.13-0.69)	1.93 (0.89-3.13)	-
T. gelada 3 – *T. gelada* 1	0.04 (0.02-0.08)	0.04 (0.01-0.12)	0.80 (0.21-1.83)	-
C. atys – (*C. torquatus* + *C. chrysogaster* + *M. sphinx* + *M. leucophaeus*)	4.19 (3.02-5.43)	4.61 (2.85-7.30)	4.87 (3.39-6.03)	-
C. torquatus – (*M. sphinx* + *C. chrysogaster* + *M. leucophaeus*)	3.59 (2.58-4.75)	3.82 (2.33-6.11)	4.29 (2.83-5.51)	-
M. sphinx – (*C. chrysogaster* + *M. leucophaeus*)	2.67 (1.88-3.67)	2.74 (1.60-4.56)	3.36 (2.05-4.52)	-
C. chrysogaster – *M. leucophaeus*	1.85 (1.13-2.74)	1.85 (0.94-3.29)	2.63 (1.49-3.73)	-
Mandrillus – *Cercocebus*	-	-	-	4.85 (3.58-6.23)
M. sylvanus – Asian macaques	5.93 (4.95-6.93)	5.93 (4.84-6.48)	6.29 (5.77-6.50)	5.12 (4.27-5.93)
(*M. silenus* + *M. tonkeana*) – remaining macaques	5.16 (4.18-6.15)	5.19 (4.13-6.01)	5.89 (5.30-6.26)	4.13 (3.26-5.01)
M. silenus – *M. tonkeana* (Sulawesi macaques)	3.34 (2.11-4.51)	3.23 (1.91-4.58)	4.58 (3.79-5.19)	3.13 (2.35-3.98)
M. thibetana – (*M. mulatta* + *M. arctoides* + *M. fascicularis*)	3.97 (3.09-4.90)	3.93 (2.90-4.98)	5.04 (4.41-5.52)	-
M. fascicularis – (*M. mulatta* + *M. arctoides*)	3.28 (2.49-4.14)	3.18 (2.22-4.20)	4.61 (3.97-5.10)	-
M. fascicularis 2 – (*M. fascicularis* 1 + *M. fascicularis* 3)	1.08 (0.76-1.51)	1.14 (0.62-1.99)	2.28 (1.61-3.00)	-
M. fascicularis 1 – *M. fascicularis* 3	0.60 (0.43-0.77)	0.65 (0.32-1.26)	1.12 (0.72-1.61)	-
M. mulatta – *M. arctoides*	2.86 (2.14-3.72)	2.70 (1.78-3.68)	4.32 (3.67-4.84)	-
M. mulatta 2 – (*M. mulatta* 1 + *M. mulatta* 3)	1.56 (0.93-2.30)	1.41 (0.74-2.35)	2.94 (2.27-3.50)	-
M. mulatta 1 – *M. mulatta* 3	0.02 (0.01-0.05)	0.02 (0.01-0.06)	0.09 (0.02-0.30)	-
M. thibetana 1 – *M. thibetana* 2	0.04 (0.01-0.07)	0.03 (0.01-0.08)	0.26 (0.04-0.97)	-
M. sylvanus 1 – *M. sylvanus* 2	0.04 (0.02-0.07)	0.04 (0.01-011)	0.36 (0.04-1.30)	-
(*M. arctoides* + *M. thibetana*) – (*M. fascicularis* + *M. mulatta*)	-	-	-	3.53 (2.69-4.47)
M. fascicularis – *M. mulatta*	-	-	-	2.77 (1.94-3.67)
M. arctoides – *M. thibetana*	-	-	-	2.38 (1.40-3.37)

Papio ursinus south – remaining baboons	2.22 (1.67-2.81)	2.01 (1.30-3.11)	3.31 (2.51-3.97)	-
(*P. ursinus* north + *P. cynocephalus* south + *P. kindae*) – remaining baboons	1.98 (1.51-2.52)	1.84 (1.21-2.83)	3.18 (2.37-3.84)	-
P. kindae – (*P. ursinus* north + *P. cynocephalus* south)	1.41 (0.99-1.86)	1.27 (0.73-2.07)	2.52 (1.78-3.17)	-
P. ursinus north – *P. cynocephalus* south	0.68 (0.37-1.08)	0.61 (0.23-0.28)	1.36 (0.83-1.92)	-
(*P. anubis* west2 + *P. papio* + *P. anubis* west1) – (*P. anubis* east + *P. hamadryas* + *P. cynocephalus* north)	1.44 (1.07-1.89)	1.29 (0.81-2.05)	2.51 (1.77-3.18)	-
(*P. anubis* west2 + *P. papio*) – *P. anubis* west1	1.18 (0.82-1.58)	1.02 (0.60-1.67)	2.21 (1.52-2.86)	-
P. anubis west2 – *P. papio*	1.10 (0.74-1.49)	0.94 (0.52-1.54)	2.13 (1.45-2.78)	-
(*P. anubis* east + *P. hamadryas* 1 + *P. hamadryas* 2) – *P. cynocephalus* north	0.40 (0.26-0.58)	0.37 (0.20-0.69)	0.89 (0.46-1.49)	-
P. hamadryas 2 – (*P. anubis* east + *P. hamadryas* 1)	0.25 (0.16-0.37)	0.21 (0.11-0.40)	0.58 (0.29-1.04)	-
P. anubis east – *P. hamadryas* 1	0.21 (0.12-0.33)	0.18 (0.09-0.35)	0.52 (0.25-0.92)	-
P. papio – (*P. anubis* + *P. hamadryas*)	-	-	-	1.21 (0.70-1.79)
P. anubis – *P. hamadryas*	-	-	-	0.72 (0.35-1.17)

[1] nuclear divergence ages from [15] based on 34,927 bp from 54 genes.

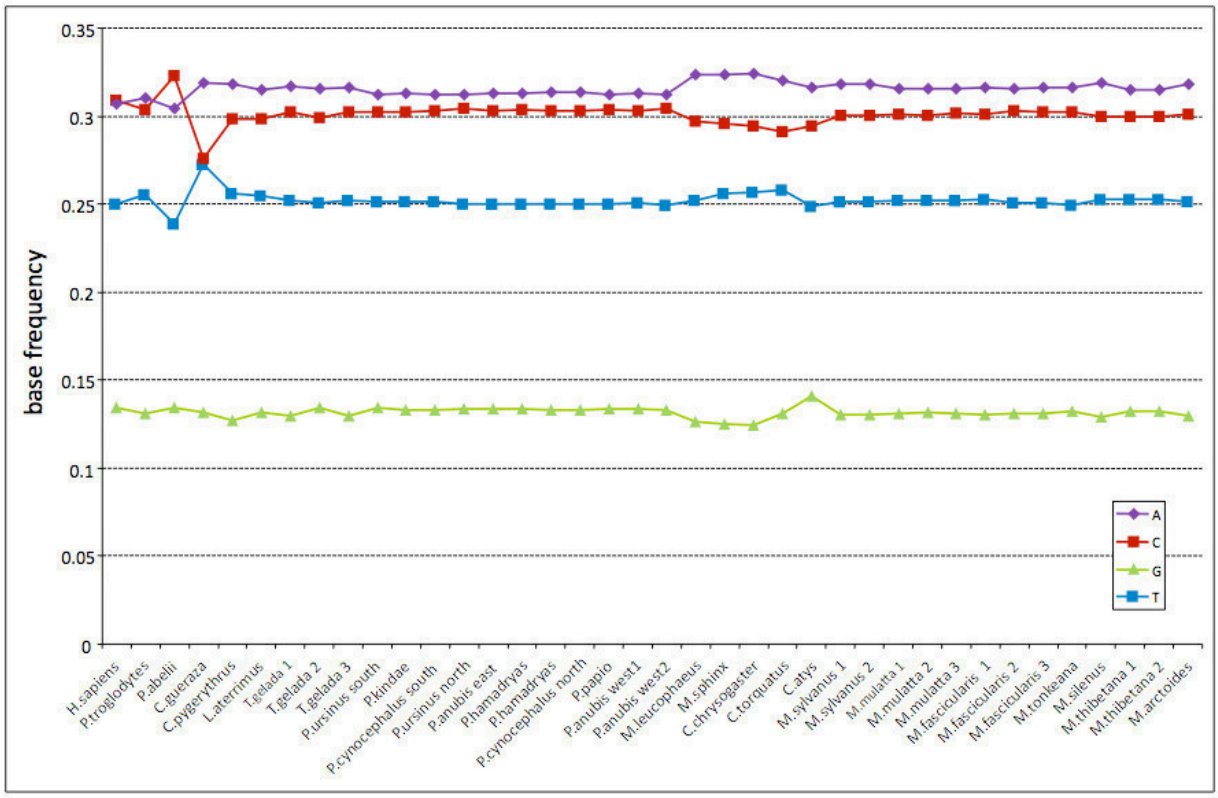

Figure S1. Nucleotide composition among Papionini and outroup taxa.

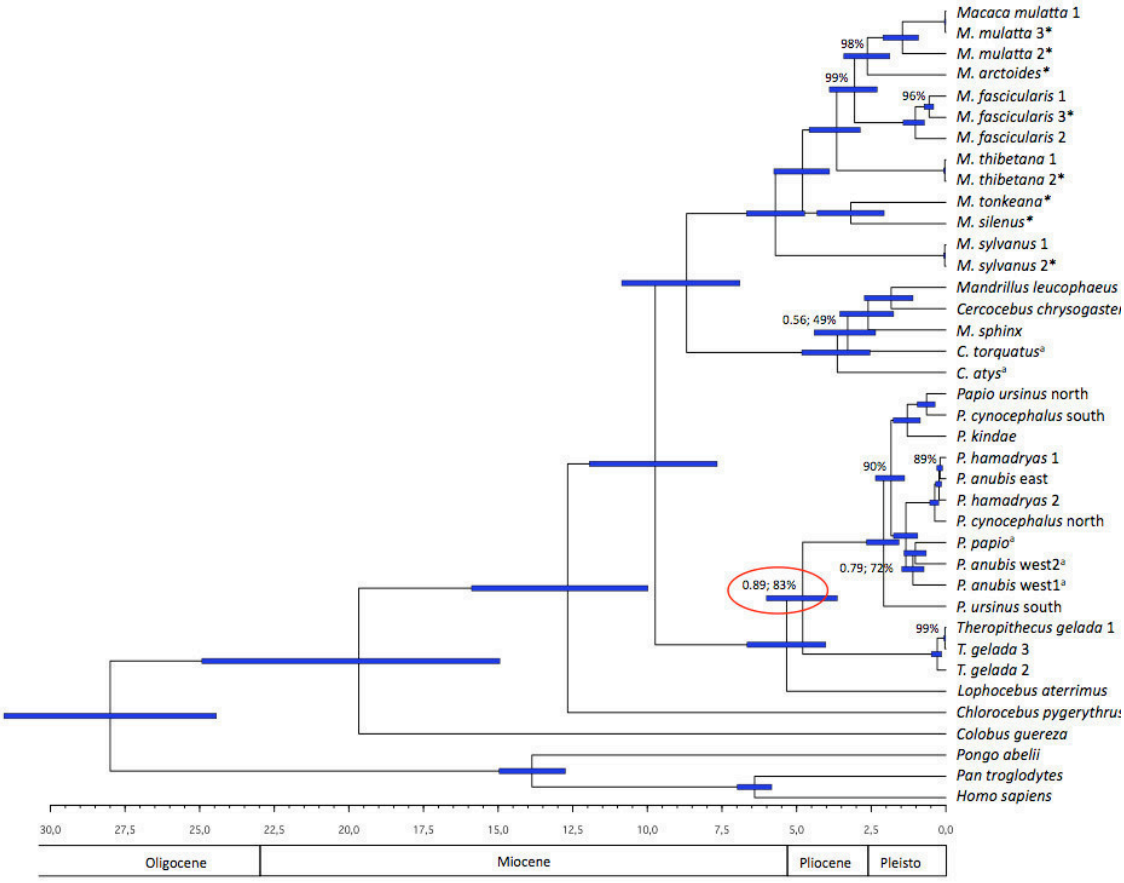

Figure S2. Ultrametric tree of Papionini and outgroup taxa as inferred from dataset 2. Tree topologies as inferred from Bayesian (MrBayes) as well as from ML (RAxML) estimations were mainly identical with some exceptions. All unlabelled branches show ML BP of 100% and Bayesian PP of 1.0. Values below are indicated at respective nodes. Taxa indicated with a are arranged differently in the ML (RAxML) and Bayesian tree (MrBayes): ((*P. anubis* west2, *P. anubis* west1) P. papio); ((*C. torquatus*, *C. atys*), ((*C. chrysogaster*, *M. leucophaeus*), *M. sphinx*)). Red ellipse indicates main difference to Figure 1. * = sequences were newly generated in this study.

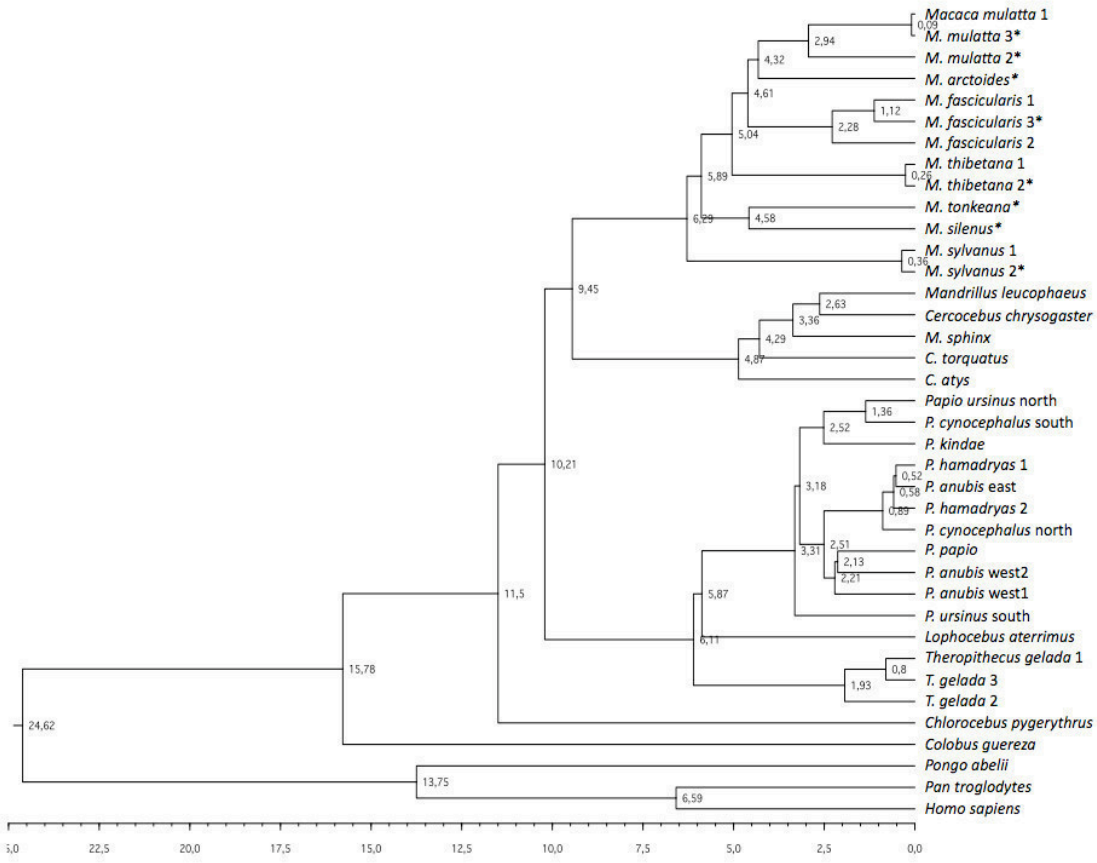

Figure S3. Tree topology including divergence dates as estimated with an auto-correlated relaxed clock model as implemented in PhyloBayes 3.3. Time scale shows million years before present. * = sequences were newly generated in this study.

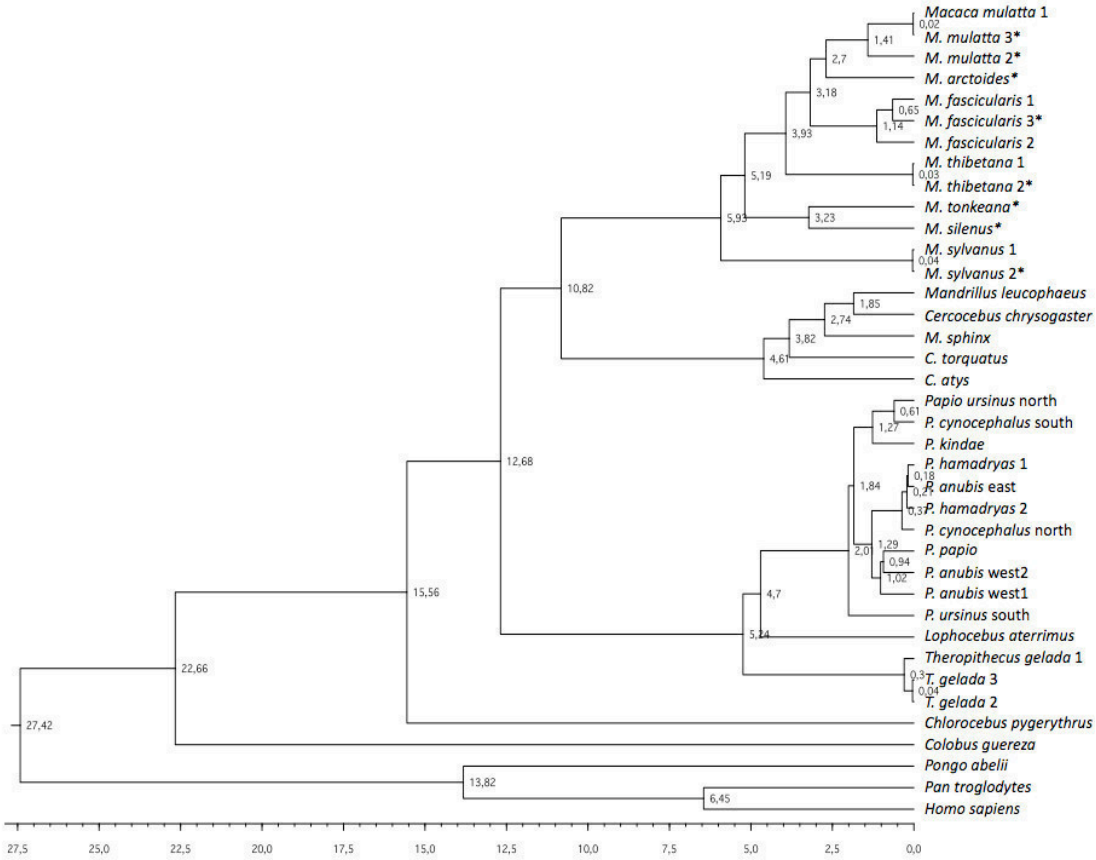

Figure S4. Tree topology including divergence dates as estimated with an uncorrelated relaxed clock model as implemented in PhyloBayes 3.3. Time scale shows million years before present. * = sequences were newly generated in this study.

Table S2. Studied species and individuals along with their GenBank accession numbers.

Species	Accession no.
Macaca sylvanus 1	AJ309865
***M. sylvanus* 2**	**KJ567054**
M. arctoides	**KJ567055**
M. fascicularis 1	FJ906803
M. fascicularis 2	KF305937
***M. fascicularis* 3**	**KJ567052**
M. mulatta 1	AY612638
***M. mulatta* 2**	**KJ567051**
***M. mulatta* 3**	**KJ567053**
M. thibetana 1	EU294187
***M. thibetana* 2**	**KJ567056**
M. tonkeana	**KJ567058**

M. silenus	**KJ567057**
Mandrillus leucophaeus	JQ257001
M. sphinx	KC757403
Cercocebus chrysogaster	KC757390
C. torquatus [1)]	JQ256999
C. atys	JQ256998
Papio anubis east	JX946196
P. anubis west 1	JX946198
P. anubis west 2	JX946197
P. cynocephalus north	JX946199
P. cynocephalus south	JX946200
P. hamadryas 2	JX946201
P. hamadryas 1	NC001992
P. papio	JX946203
P. kindae	JX946202
P. ursinus north	JX946204
P. ursinus south	JX946205
Theropithecus gelada 1	FJ785426
T. gelada 2	JQ257000
T. gelada 3	KC757412
Lophocebus aterrimus	KC757401
Chlorocebus pygerythrus	EF597500
Colobus guereza	AY863427
Pongo abelii	X97707
Pan troglodytes	D38113
Homo sapiens	X93334

[1]) designated as *Lophocebus albigena* in [63], but species identification most likely wrong. Sequences in bold were newly generated in this study.

Chapter 3 no supplementary material

Chapter 4

Table SI. Detailed information about studied mtDNA genomes (geographical origin, source, GenBank accession number, sequencing method).

species/individual no.	location	coordinates	source	source ID	accession no.	sequencing method
M. fascicularis 1	mainland Southeast Asia according to haplotype	unknown	GenBank		FJ906803	
M. fascicularis 2	mainland Southeast Asia according to haplotype	unknown	GenBank		KF305937	
M. fascicularis 3	Vietnam	unknown	GenBank/ Covance		KJ567052	
***M. fascicularis* 4**	Bangkok, Thailand	ca. 13°45'N, 100°31'E	ZSM	1904/05	KM851024	Ion Torrent
***M. fascicularis* 5**	Bangkok, Thailand	ca. 13°45'N, 100°31'E	ZSM	1949/1486	KM851036	Ion Torrent

***M. fascicularis* 6**	Tanah Merah, Kelantan, West Malaysia	ca. 5°48'N, 102°09'E	MdZain	Tanah Merah1	KM851016	Ion Torrent
***M. fascicularis* 7**	Terengganu, Pulau Redang, West Malaysia	ca. 5°47'N, 103°01'E	MdZain	Redang1	KM851012	Sanger
***M. fascicularis* 8**	Kuala Gula, Perak, West Malaysia	ca. 4°57'N, 100°32'E	MdZain	Kuala Gula1	KM851018	Ion Torrent
***M. fascicularis* 9**	Sepang, Selangor, West Malaysia	ca. 2°49'N, 101°44'E	MdZain	Sepang1	KM851017	Ion Torrent
***M. fascicularis* 10**	Kluang, Johor, West Malaysia	ca. 2°02'N, 103°19'E	MdZain	Kluang1	KM851015	Ion Torrent
***M. fascicularis* 11**	Batang Kwis, Medan, Sumatra	ca. 3°40'N, 98°44'E	ZSM	1906/35	KM850999	Sanger
***M. fascicularis* 12**	Deli, Sumatra	ca. 3°35'N, 98°40'E	ZSM	1921/183	KM851010	Ion Torrent
***M. fascicularis* 13**	Deli, Sumatra	ca. 3°35'N, 98°40'E	ZSM	1921/187	KM851011	Ion Torrent
***M. fascicularis* 14**	Kampong Baru, Sumatra	ca. 3°33'N, 98°41'E	ZSM	1906/61	KM850998	Sanger
***M. fascicularis* 15**	Sungei Rampalz Serdang, east coast Sumatra	ca. 3°30'N, 99°00'E	ZSM	1905/135	KM851022	Ion Torrent
***M. fascicularis* 16**	Sungei Rampalz Serdang, east coast Sumatra	ca. 3°30'N, 99°00'E	ZSM	1905/134	KM851035	Ion Torrent
***M. fascicularis* 17**	Simpang Bangka, Sumatra	ca. 2°18'S, 106°04'E	ZSM	1905	KM851023	Ion Torrent
***M. fascicularis* 18**	Simpang Bangka, Sumatra	ca. 2°18'S, 106°04'E	ZSM	none	KM851034	Ion Torrent
***M. fascicularis* 19**	Kediri, Java	ca. 7°48'S, 112°15'E	ZSM	1911/2363	KM851031	Ion Torrent
***M. fascicularis* 20**	Kediri, Java	ca. 7°48'S, 112°15'E	ZSM	1911/2364	KM851021	Sanger + Ion Torrent
***M. fascicularis* 21**	Kediri, Java	ca. 7°48'S, 112°15'E	ZSM	1911/2365	KM851029	Ion Torrent
***M. fascicularis* 22**	Kediri, Java	ca. 7°48'S, 112°15'E	ZSM	1911/2356	KM851020	Ion Torrent
***M. fascicularis* 23**	Kediri, Java	ca. 7°48'S, 112°15'E	ZSM	1911/2353	KM851028	Ion Torrent
***M. fascicularis* 24**	Lelogama, Timor	ca. 9°44'S, 123°57'E	ZSM	1911/2104	KM851033	Ion Torrent
***M. fascicularis* 25**	Lelogama, Timor	ca. 9°44'S, 123°57'E	ZSM	1911/2103	KM851026	Ion Torrent
***M. fascicularis* 26**	Lelogama, Timor	ca. 9°44'S, 123°57'E	ZSM	1911/2102	KM851025	Ion Torrent
***M. fascicularis* 27**	Lelogama, Timor	ca. 9°44'S, 123°57'E	ZSM	1911/2105	KM851019	Ion Torrent
***M. fascicularis* 28**	Lelogama, Timor	ca. 9°44'S, 123°57'E	ZSM	1911/2106	KM851027	Ion Torrent
***M. fascicularis* 29**	Lelogama, Timor	ca. 9°44'S, 123°57'E	ZSM	1911/2107	KM851037	Ion Torrent
***M. fascicularis* 30**	Kuatnana, Timor	ca. 9°50'S, 124°10'E	ZSM	1911/2108	KM851030	Ion Torrent
***M. fascicularis* 31**	Bokong, Timor	ca. 9°58'S, 124°04'E	ZSM	1911/2109	KM851032	Sanger + Ion Torrent
***M. fascicularis* 32**	Lundu, Sarawak, Borneo	ca. 1°40'N, 109°48'E	MdZain	Lundu1	KM851014	Sanger
***M. fascicularis* 33**	Sangan, west coast Borneo	unknown	ZSM	1910/1373	KM851005	Ion Torrent
***M. fascicularis* 34**	Sangan, west coast Borneo	unknown	ZSM	1909/1358	KM851007	Ion Torrent
***M. fascicularis* 35**	Paian, west coast Borneo	unknown	ZSM	1909/1387	KM851009	Ion Torrent
***M. fascicularis* 36**	Sungei, Malai, west coast Borneo	unknown	ZSM	1910/1437	KM851003	Ion Torrent
***M. fascicularis* 37**	Baeajan, west coast Borneo	unknown	ZSM	1910/1534	KM851004	Ion Torrent
***M. fascicularis* 38**	Plandjan, central Borneo	ca. 0°04'N, 111°30'E	ZSM	1909/766	KM851002	Sanger

***M. fascicularis* 39**	central Borneo	ca. 0°04'N, 111°30'E	ZSM	1909/771	KM851008	Ion Torrent
***M. fascicularis* 40**	Schkadon, central Borneo	ca. 0°04'N, 111°30'E	ZSM	1909/788	KM851006	Ion Torrent
***M. fascicularis* 41**	Tawau Hill Park, Sabah, Borneo	ca. 4°27'N, 117°57'E	MdZain	Tawau Hill1	KM851013	Sanger
***M. fascicularis* 42**	Philippines	unknown	Covance	none	KM851001	Sanger
***M. fascicularis* 43**	Mauritius	unknown	DPZ	23145	KM851000	Sanger
M. sylvanus 1			GenBank		AJ309865	
M. sylvanus 2			GenBank		KJ567054	
M. arctoides			GenBank		KJ567055	
M. mulatta 1			GenBank		AY612638	
M. mulatta 2	China		GenBank		KJ567051	
M. mulatta 3	India		GenBank		KJ567053	
M. thibetana 1			GenBank		EU294187	
M. thibetana 2			GenBank		KJ567056	
M. tonkeana			GenBank		KJ567058	
M. silenus			GenBank		KJ567057	
Theropithecus gelada			GenBank		FJ785426	
Papio hamadryas			GenBank		NC001992	
Chlorocebus pygerythrus			GenBank		EF597500	
Colobus guereza			GenBank		AY863427	
Pongo abelii			GenBank		X97707	
Pan troglodytes			GenBank		D38113	
Homo sapiens			GenBank		X93334	
M. fascicularis individuals in bold were newly generated for this study						
ZSM = Bavarian State Collection of Zoology						
DPZ = German Primate Center						

Table SII. Estimated divergence ages in Ma and 95 % credibility intervals (in parentheses) among lineages.

split	divergence age (Ma)
Cercopithecidae - Hominidae	28.60 (25.31-31.78)
Pongo - *Homo* + *Pan*	13.82 (12.68-14.86)
Homo - *Pan*	6.32 (5.73-6.89)
Colobus - Cercopithecinae	19.89 (16.17-23.87)
Chlorocebus - Papionini	12.81 (10.59-15.22)
Theropithecus + *Papio* - *Macaca*	10.90 (8.92-12.90)
Theropithecus - *Papio*	4.77 (3.87-5.72)
Macaca sylvanus - other *Macaca*	6.10 (5.23-6.92)
Macaca sylvanus 1 - *M. sylvanus* 2	0.04 (0.02-0.07)
Macaca silenus + *M. tonkeana* - other *Macaca*	5.49 (4.69-6.34)
Macaca silenus - *M. tonkenana*	3.70 (2.80-4.54)
Macaca thibetana - other *Macaca*	4.16 (3.47-4.85)
Macaca thibetana 1 - *M. thibetana* 2	0.04 (0.02-0.06)
Macaca arctoides + *M. mulatta* - *M. fascicularis*	3.42 (2.83-4.01)
M. arctoides - *M. mulatta*	3.02 (2.42-3.60)
Macaca mulatta 2 - *M. mulatta* 1 + *M. mulatta* 3	1.67 (1.19-2.15)
Macaca mulatta 1 - *M. mulatta* 3	0.02 (0.01-0.04)
Macaca fascicularis Clade A - *M. fascicularis* Clade B	1.70 (1.36-2.04)
Clade A: MFAS2+4+5 - MFAS1+3+6-16	0.96 (0.78-1.16)
Clade A: MFAS2 - MFAS4+5	0.30 (0.20-0.41)
Clade A: MFAS4 - MFAS5	0.04 (0.02-0.07)
Clade A: MFAS1+3 - MFAS6-16	0.88 (0.70-1.06)
Clade A: MFAS1 - MFAS3	0.45 (0.34-0.57)
Clade A: MFAS7 - MFAS6+8-16	0.70 (0.55-0.84)
Clade A: MFAS6 - MFAS8-16	0.56 (0.43-0.68)
Clade A: MFAS8 - MFAS9-16	0.40 (0.31-0.49)
Clade A: MFAS9 - MFAS10-16	0.27 (0.21-0.34)
Clade A: MFAS10+14 - MFAS11-13+15+16	0.25 (0.20-0.31)
Clade A: MFAS10 - MFAS14	0.22 (0.16-0.28)
Clade A: MFAS11-13 - MFAS15+16	0.20 (0.15-0.25)
Clade A: MFAS11 - MFAS12+13	0.11 (0.07-0.15)
Clade A: MFAS12 - MFAS13	0.02 (0.01-0.04)
Clade A: MFAS15 - MFAS16	0.04 (0.02-0.07)
Clade B: MFAS24-31 - MFAS17-23+32-43	0.93 (0.74-1.12)
Clade B: MRCA MFAS24-31	0.03 (0.01-0.04)
Clade B: MFAS19-23 - MFAS17+18+32-43	0.87 (0.70-1.05)
Clade B: MRCA MFAS19-23	0.01 (0.00-0.02)
Clade B: MFAS43 - MFAS17+18+32-42	0.84 (0.67-1.02)
Clade B: MFAS17+18 - MFAS32-42	0.61 (0.47-0.75)
Clade B: MFAS17 - MFAS18	0.18 (0.11-0.25)
Clade B: MFAS41+42 - MFAS32-40	0.33 (0.26-0.41)
Clade B: MFAS41 - MFAS42	0.21 (0.15-0.28)
Clade B: MFAS37-39 - MFAS32-35+40	0.30 (0.24-0.38)
Clade B: MFAS37 - MFAS38+39	0.05 (0.03-0.07)
Clade B: MFAS38 - MFAS39	0.03 (0.02-0.05)
Clade B: MFAS32+36 - MFAS33-35+40	0.28 (0.22-0.35)
Clade B: MFAS32 - MFAS36	0.20 (0.14-0.26)
Clade B: MFAS35 - MFAS3/34+40	0.09 (0.06-0.13)
Clade B: MFAS33/34 - MFAS40	0.04 (0.02-0.07)

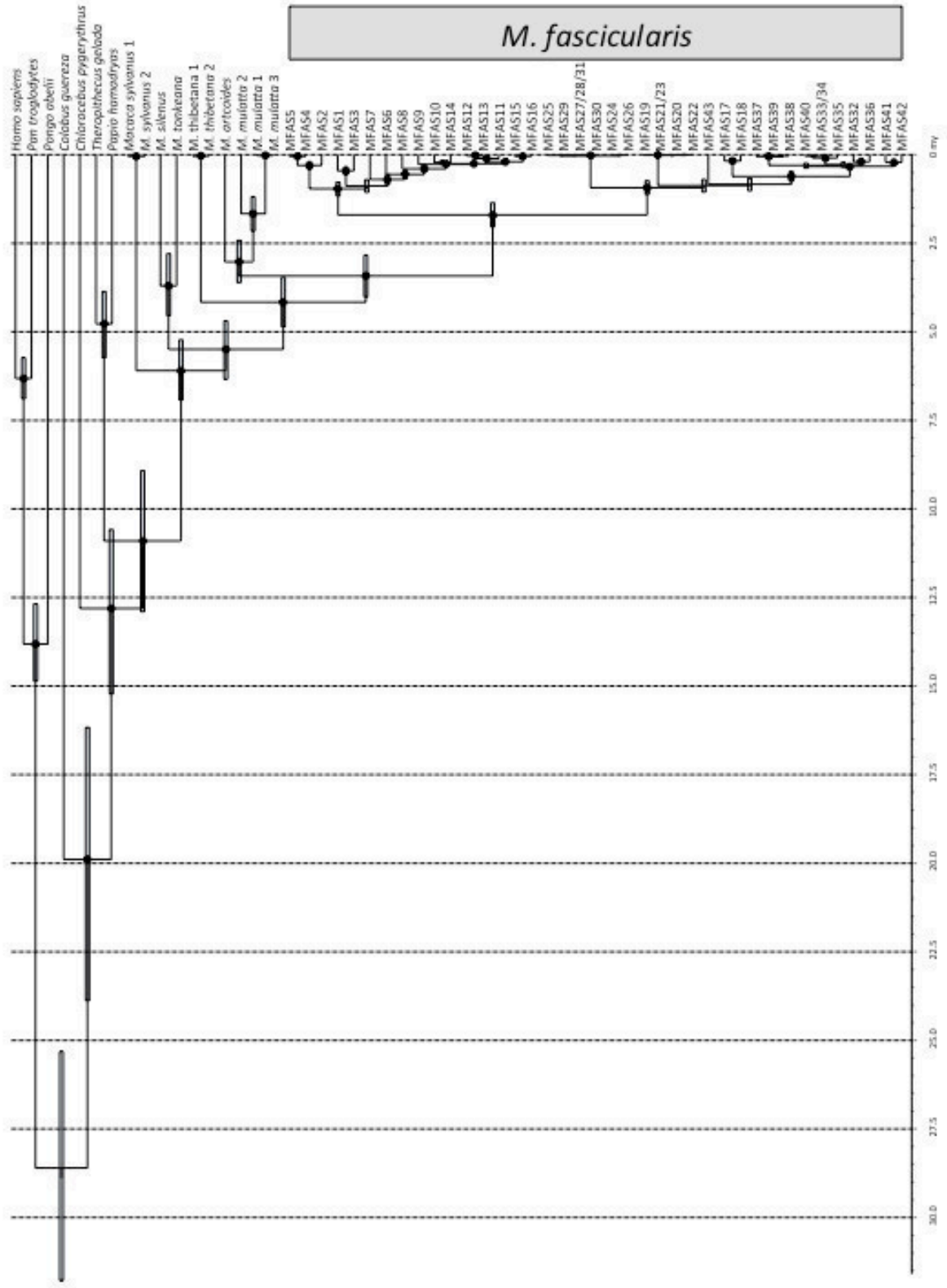

Figure SI. Ultrametric tree showing phylogenetic relationships and divergence ages among 56 unique mtDNA genome sequences. Grey bars indicate 95 % credibility intervals of divergence times and the time scale below shows million years before present. Numbers correspond to IDs in Figs. 1 & 2 and Supporting Information Table SI. Black dots indicate ML bootstrap support of >95 % and Bayesian posterior probabilities of 1.0.

Danksagung

Zuerst möchte ich mich herzlich bei Christian Roos und Dietmar Zinner für die geduldige Betreuung und die tatkräftige Unterstützung dieser Doktorarbeit bedanken. Ohne sie wäre diese Arbeit nicht zustande gekommen.

Besonderer Dank geht auch an Christiane Schwarz für die tolle Zusammenarbeit im Labor. Ihr Einsatz hat mir viel Arbeit abgenommen.

Des Weiteren gilt mein Dank Lutz Walter, Markus Brameier, Nico Westphal, Kai Böker, Tanja Haus, Gisela Kopp und allen Mitarbeitern der Abteilung Primatengenetik für ihre Unterstützung und die gute Zusammenarbeit. Hier möchte ich mich besonders bei Jakob Kolleck bedanken, der durch seinen Einsatz im ancient DNA Labor wichtige Daten für diese Arbeit generiert hat. An dieser Stelle sei auch Sabine Hutschenreuther gedankt, die mir geholfen hat, die vielen Makaken in der Zoologischen Staatssammlung München zu beproben. Außerdem ein großes Dankeschön an Rebecca Jürgens für Tipps und Korrekturen sowie motivierende Worte. Besonders bedanken möchte ich mich auch dafür, dass ich im Rahmen meiner Arbeit in der Primatengenetik die Möglichkeit bekommen habe, nach Asien zu reisen und neben Konferenzbesuchen auch einige Primaten in ihrem natürlichen Lebensraum sehen zu können.

Zum Schluss möchte ich natürlich auch meiner Familie, meinen Freunden und besonders Verena danken, die mich immer unterstützt und motiviert haben, auch wenn ich in den letzten Monaten sicher nicht immer leicht zu ertragen war.

Lebenslauf

Rasmus Liedigk
Diplom Biologe
Geboren am 19.07.1978 in Westerstede
Staatsangehörigkeit: deutsch

08/2010 – 09/2014	Anfertigung der vorliegenden Dissertation in der Abteilung Primatengenetik am Deutschen Primatenzentrum Göttingen
11/2009 – 07/2010	Wissenschaftlicher Mitarbeiter am Johann-Friedrich-Blumenbach Institut für Zoologie, Georg-August-Universität Göttingen.
12/2008 – 10/2009	Anfertigung der Diplomarbeit „Molecular phylogeny of species groups within the genera *Trachypithecus* and *Semnopithecus*“ am Deutschen Primatenzentrum Göttingen
10/2003 – 10/2008	Studium im Fachbereich Biologie, Georg-August-Universität Göttingen Schwerpunkte: Zoologie, Evolutionsbiologie, Naturschutz, Geologie, Abschluss: Diplom

Eidesstattliche Erklärung

Ich erkläre hiermit, dass ich diese Dissertation selbstständig 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.

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, 4.9. 2014