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Comparing mitogenomic timetrees for two African savannah primate genera (*Chlorocebus* and *Papio*)

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Complete mitochondrial (mtDNA) genomes have proved to be useful in reconstructing primate phylogenies with higher resolution and confidence compared to reconstructions based on partial mtDNA sequences. Here, we analyse complete mtDNA genomes of African green monkeys (genus *Chlorocebus*), a widely distributed primate genus in Africa representing an interesting phylogeographical model for the evolution of savannah species. Previous studies on partial mtDNA sequences revealed nine major clades, suggesting several cases of para- and polyphyly among *Chlorocebus* species. However, in these studies, phylogenetic relationships among several clades were not resolved, and divergence times were not estimated. We analysed complete mtDNA genomes for ten *Chlorocebus* samples representing major mtDNA clades to find stronger statistical support in the phylogenetic reconstruction than in the previous studies and to estimate divergence times. Our results confirmed para- and polyphyletic relationships of most *Chlorocebus* species, while the support for the phylogenetic relationships between the mtDNA clades increased compared to the previous studies. Our results indicate an initial west–east division in the northern part of the *Chlorocebus* range with subsequent divergence into north-eastern and southern clades. This phylogeographic scenario contrasts with that for another widespread African savannah primate genus, the baboons (*Papio*), for which a dispersal from southern Africa into East and West Africa was suggested.

ADDITIONAL KEYWORDS: African green monkeys – baboons – mitochondrial genomes – phylogeny – phylogeography.

INTRODUCTION

The availability and analyses of genetic data have a tremendous impact on the understanding of phylogenetic relationships and evolutionary history of organisms, at which different genetic markers (mitochondrial, autosomal or gonosomal) or respective whole genomes can provide insights into different aspects of the evolutionary history of taxa (Moore, 1995; Hoelzer, 1997; Maddison, 1997; Nichols, 2001; Funk & Omland, 2003; Avise, 2004). It has been shown that phylogenies based on mitochondrial and different nuclear markers are often incongruent, due to sex-biased dispersal, nuclear swamping or mitochondrial capture after introgression (Moore, 1995; Hoelzer, 1997; Maddison, 1997; Avise, 2004; Roos *et al.*, 2011; Zinner, Arnold & Roos, 2011a; Liedigk *et al.*, 2012; Wang *et al.*, 2012; Haus, Roos & Zinner, 2013a). Moreover, in taxa with female-biased dispersal, as in the majority of mammal species including primates (Greenwood, 1980), the mitogenomic population structure often reveals

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a geographic pattern not recognizable if nuclear markers alone are examined (Hoelzer, 1997; Funk & Omland, 2003; Avise, 2004). The mitogenomic analysis is of particular interest in widely distributed taxa, for instance among primates, baboons (*Papio*) and African green monkeys (*Chlorocebus*), which range over large parts of sub-Saharan Africa.

The genus *Chlorocebus* is one of the most widely distributed primate genera in Africa. African green monkeys are primarily found in the sub-Saharan savannah biome (Lernould, 1988; Kingdon, 1997; Anandam *et al.*, 2013) (Fig. 1). The genus comprises six major taxa, which have previously been recognized as forms of the *aethiops* group within the genus *Cercopithecus* (Hill, 1966; Napier, 1981; Grubb *et al.*, 2003). Based on recent morphological and genetic studies, they are now grouped into their own genus *Chlorocebus*, which has closest affinities to two other more terrestrial members of the Cercopithecini, *Erythrocebus* and *Allochrocebus* (Groves, 2001, 2005; Tosi *et al.*, 2002; Xing *et al.*, 2007; Perelman *et al.*, 2011; Springer *et al.*, 2012). The taxonomy within the genus is still disputed (Groves & Kingdon, 2013). Some authors consider *Chlorocebus aethiops* as a polytypic species comprising five or six subspecies (Kingdon, 1997; Grubb *et al.*, 2003; Elton, Dunn & Cardini, 2010), whereas others recognize six parapatric species: *C. aethiops* (grivet), *C. djamdjamensis* (Bale monkey), *C. sabaeus* (West African green monkey), *C. cynosuros* (malbrouck monkey), *C. tantalus* (tantalus monkey) and *C. pygerythrus* (vervet monkey) (Groves, 2001; Anandam *et al.*, 2013; Haus *et al.*, 2013b). Subspecies have been described in *C. aethiops*, *C. tantalus* and *C. pygerythrus* (Groves, 2001; Butynski & Kingdon, 2013; Isbell & Jaffe, 2013; Nakagawa, 2013), whereas the other three members of *Chlorocebus* are regarded as monotypic.

Only few studies have been conducted on phylogenetic relationships within *Chlorocebus*. Although complete mitochondrial (mtDNA) genomes were analysed in a study by Wertheim & Worobey (2007), a total of just six individuals of four taxa (i.e. *C. aethiops*, *C. sabaeus*,

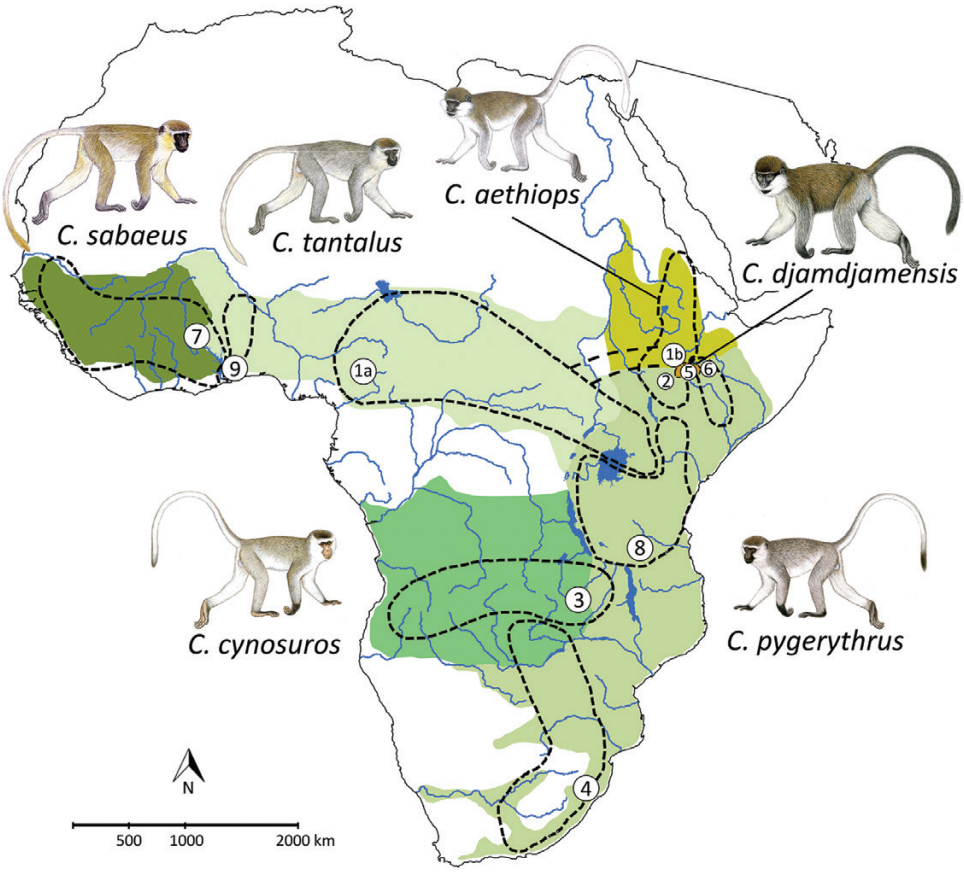

Figure 1. Distribution of the six *Chlorocebus* species and the nine major mtDNA haplogroups (1–9; Haus *et al.*, 2013b). Coloured species distributions are modified from Lernould (1988) and Kingdon (1997). Dashed lines indicate the distribution of the nine major mtDNA haplogroups, and circles indicate the geographic provenance of samples used in this study (see Table S1). Green monkey drawings by Stephen Nash.

C. tantalus and *C. pygerythrus*) were included in their study, and the geographical provenance of only two samples was known. Another mitogenomic study did not include *C. djamdjamensis* (Guschanski *et al.*, 2013). Recently, the first *Chlorocebus* phylogeny based on nuclear genomic data was presented by Warren *et al.* (2015), but *C. djamdjamensis* was also lacking in the study.

The most geographically broad sampling including all taxa was conducted by Haus *et al.* (2013b), who analysed mitochondrial cytochrome *b* (cytb) gene sequences, including samples covering almost the entire range of the genus. This study revealed nine major mtDNA clades that reflect geographic distributions rather than morphological taxa (Haus *et al.*, 2013b). However, monophyly of several clades was not well supported, and phylogenetic relationships among them could not be resolved, suggesting that cytb sequence information alone might not be sufficient for resolving the apparently rapid radiation of African green monkeys' mtDNA lineages.

However, applying complete mtDNA genomes has improved confidence in reconstructed primate phylogenies compared to shorter mtDNA fragments (Roos *et al.*, 2011; Liedigk *et al.*, 2012; Finstermeier *et al.*, 2013; Guschanski *et al.*, 2013; Zinner *et al.*, 2013; Pozzi *et al.*, 2014). In our study, we therefore provide new complete mtDNA genomes of *Chlorocebus* and base our phylogeographic analysis on these data.

The observed discordances between mtDNA haplogroups and morphological phenotypes of *Chlorocebus* are not unique; rather, they resemble those reported from another widespread African savannah primate genus, *Papio* baboons (Zinner *et al.*, 2009, 2011b, 2013; Keller *et al.*, 2010; Fig. 2). Both genera are primarily found in savannah woodland and have largely overlapping geographical distributions (Figs 1, 2). The similarity in habitat preference and distribution of *Chlorocebus* and *Papio* makes it likely that the evolutionary histories of both genera were influenced by similar climatic and geological events, while potential differences in divergence times and population histories of the genera might indicate taxon-specific responses to environmental changes.

In this study, we analyse complete mtDNA genomes of ten *Chlorocebus* samples representing the nine mtDNA clades revealed by Haus *et al.* (2013b) and compare the resulting phylogenetic relationships with

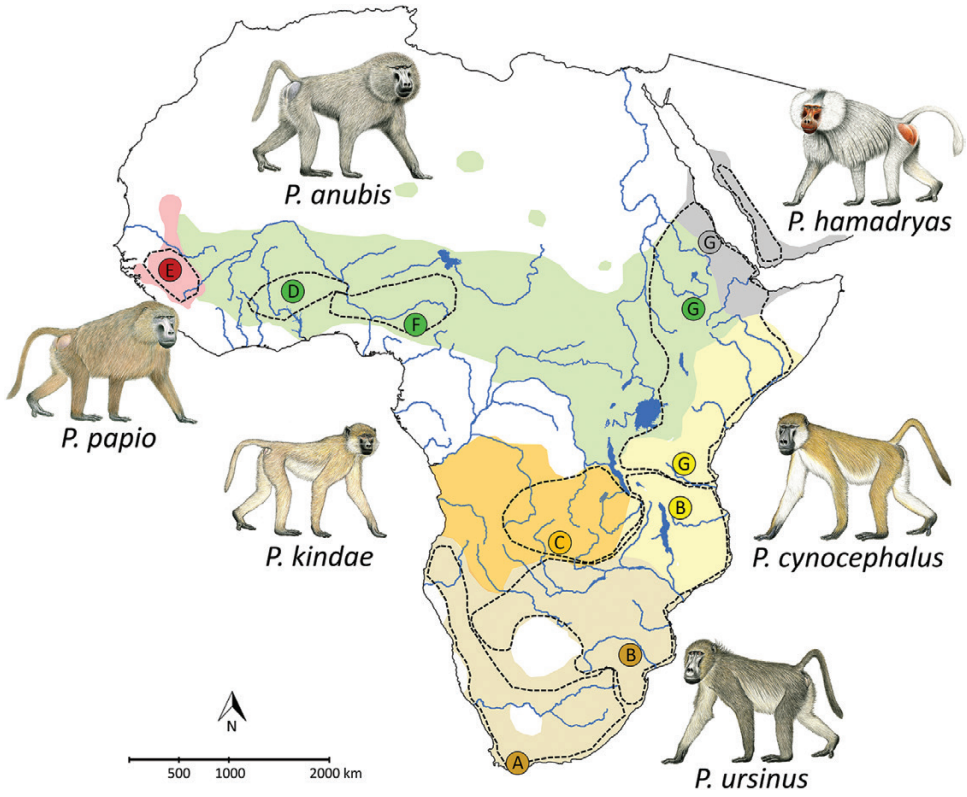

Figure 2. Distribution of the six *Papio* species and the seven major mtDNA haplogroups (A–G; Zinner *et al.*, 2009, 2013). Species distributions are modified from Zinner *et al.* (2013). Dashed lines indicate the distribution of the seven major mtDNA haplogroups, and circles indicate the geographic provenance of samples used in this study (see Table S1). Baboon drawings by Stephen Nash.

a morphology-derived phylogeny (Groves, 2001, 2005) and divergence times to those based on partial mtDNA sequence data (Haus *et al.*, 2013b), Y-chromosomal DNA (Haus *et al.*, 2013a) and nuclear genome data (Warren *et al.*, 2015). We expect that the analysis of complete mtDNA genomes leads to a better resolution of the phylogenetic relationships among lineages than in the previous studies based on partial mtDNA sequence data. We also include complete mtDNA genomes of *Papio* (Zinner *et al.*, 2013) in our phylogenetic analysis and compare branching patterns and divergence times of *Chlorocebus* taxa with those of *Papio* taxa in order to evaluate the potential role of climatic events in their radiations and to investigate general phylogeographic trends for African savannah mammals.

MATERIAL AND METHODS

Sampling

We used faecal samples of wild African green monkeys originating from ten sites in Ethiopia, Tanzania, Zambia, Ghana, Nigeria and the Republic of South Africa (RSA). These samples represent the nine mtDNA clades revealed by Haus *et al.* (2013b) (Fig. 1, Supporting Information Table S1). The samples were collected between 2005 and 2010 by Haus *et al.* (2013b). Faecal samples were preserved in > 90% ethanol for ≥ 24 h, dried and transferred into tubes with silica beads for further storage (Nsubuga *et al.*, 2004). Sample collection was conducted in compliance with the animal care regulations and the principles of the American Society of Primatologists for the ethical treatment of nonhuman primates. All samples were collected from wild, non-habituated groups without threatening or harming the subjects. The research complied with protocols approved by the German Primate Center and adhered to the legal requirements of the countries in which samples were collected.

DNA extraction, amplification and sequencing

We extracted total genomic DNA from faecal samples with the First-DNA all-tissue kit (Gen-Ial, Troisdorf, Germany), following the standard protocol as provided by the company with minor changes. The volume of lysis buffers 1, 2 and 3 was increased to 1000, 100 and 600 µL, respectively, the volume of proteinase K was increased from 10 to 20 µL and an additional chloroform separation step was used. Extracted DNA was dissolved in 50 µL molecular-grade water. Until further processing, DNA extracts were stored at –20 °C.

Since DNA extracted from faecal samples is usually degraded, we amplified complete mtDNA genomes via 23 fragments with sizes of 1–1.2 kb and an overlap of 100–300 bp (primer information is available upon request). We used 1 U BiothermTaq 5000 (Genecraft, Cologne, Germany) in a 30 µL polymerase chain reaction (PCR) mix (1× reaction buffer, 0.16 mM of each dNTP, 0.33 µM of each primer) with a wax-mediated hot-start technique and with the following thermocycler conditions: 94 °C for 2 min, followed by 40 cycles of 94 °C for 1 min, 50–52 °C for 1 min, 72 °C for 1.5 min and 72 °C for 5 min. As template, *c.* 100 ng total genomic DNA was added. We conducted all PCRs with at least one PCR blank (molecular-grade water). We ran and checked PCR products on 1% agarose gels, excised DNA fragments of relevant lengths and purified PCR products with the QIAquick Gel Extraction Kit (Qiagen, Hilden, Germany). Sequencing was performed on an ABI 3130xl sequencer using the BigDye Terminator Cycle Sequencing Kit (Applied Biosystems, Foster City, CA, USA) and the respective forward and reverse primers.

Since in some species the hypervariable region II of the control region contains two or even three homopolymers, direct sequencing of PCR products from both ends revealed incomplete sequences. Thus, respective PCR products were cloned into the pGEM-T Easy vector following the manufacturer's instructions (Promega, Madison, WI, USA) and transferred into One Shot TOP10 Electrocomp *Escherichia coli* cells (Invitrogen, Carlsbad, CA, USA). Plasmid DNA was extracted with the QIAprep Spin Miniprep Kit (Qiagen, Hilden, Germany) and sequenced using M13 primers.

Phylogenetic analyses

We checked sequence electropherograms with 4Peaks 1.8 (www.nucleobytes.com) and assembled mtDNA genomes with SeaView 4.5.3 (Gouy, Guindon & Gascuel, 2010). Annotation was performed with DOGMA (Wyman, Jansen & Boore, 2004) and manually verified, and protein-coding genes were checked for correct and complete translation with ORF Finder (www.ncbi.nlm.nih.gov/gorf/gorf.html).

For phylogenetic reconstructions, we expanded our data set with 53 additional mtDNA genomes of Catarrhini taxa derived from GenBank: five additional African green monkeys, five other Cercopithecini, 23 Papionini, ten colobines, three gibbons and seven hominids (Table S1). We included only mtDNA genomes from GenBank that were complete and had fewer than ten ambiguous sites. Sequences were aligned with Muscle 3.8.31 (Edgar, 2010) as implemented in AliView 1.18 (Larsson, 2014). We removed indels and poorly aligned positions with Gblocks 0.91b (Castresana, 2000) using standard settings. Maximum-likelihood (ML) and

Bayesian approaches were applied to reconstruct phylogenetic trees using IQ-TREE 1.3.13 (Nguyen *et al.*, 2015) and MrBayes 3.2.6 (Ronquist *et al.*, 2012), respectively. The TIM3 + I + G model was selected as the appropriate model of nucleotide substitution with IQ-TREE and jModelTest 2.1.7 (Dariba *et al.*, 2012) using the Akaike information criterion and the Bayesian information criterion. For Bayesian tree reconstructions, we conducted four independent Markov chain Monte Carlo runs with default temperature of 0.2. We ran all repetitions for 1 million generations with tree and parameter sampling in every 100 generations. We discarded the first 25% of samples as burn-in, resulting in 7501 trees per run. To check the adequacy of the burn-in and convergence of all parameters, we used the uncorrected potential scale reduction factor (Gelman & Rubin, 1992) as calculated by MrBayes and visually inspected the trace of the parameters across generations using the software Tracer 1.6.0 (http://beast.bio.ed.ac.uk/Tracer). We applied AWTY (Nylander *et al.*, 2008) to check whether posterior clade probabilities were also converging. We calculated posterior probabilities (PP) for each split and a phylogram with mean branch lengths from the posterior density of trees. The ML analysis was run with 1000 ultrafast bootstrap (BS) replications (Minh, Nguyen & von Haeseler, 2013).

We estimated divergence times using a Bayesian approach implemented in the BEAST 2.3.2 package (Bouckaert *et al.*, 2014), assuming a relaxed lognormal clock model of lineage variation (Drummond *et al.*, 2006). Eight independent analyses were conducted with varying combinations of substitution models (TrN + I + G or GTR + I + G), tree prior models (Yule or Birth–Death) and two slightly differing fossil-based calibration sets (Table 1). Calibration points in Set 1 were taken from Pozzi *et al.* (2014), while those applied in Set 2 were derived from Liedigk *et al.* (2014, 2015). The rationales to use these calibration points and a discussion of fossils supporting respective nodes are provided in Liedigk *et al.* (2014, 2015) and Pozzi *et al.* (2014). Each analysis was run for 25 million generations, with tree and parameter sampling occurring every 1000 generations. To assess the adequacy of the burn-in and convergence of all parameters, we visually inspected the trace of the parameters across generations using Tracer. We combined sampling distributions of multiple independent replicates with LogCombiner 2.3.2 and summarized and visualized

Table 1. Details about applied calibration sets (Myr = million years)

Split	Setting (Myr)	Median (95% prior distribution, Myr)	References
Calibration Set 1			Pozzi *et al.* (2014)
Cercopithecoidea – Hominoidea	Uniform; lower 25.2; upper 34.0	29.6 (25.4–33.8)	Seiffert *et al.* (2005) and; Stevens *et al.* (2013)
Pongo – *Gorilla* / *Pan* / *Homo*	Uniform; lower 12.5; upper 18.0	15.3 (12.6–17.9)	Kelley (2002)
Homo – *Pan*	Uniform; lower 5.0; upper 10.0	7.5 (5.1–9.9)	Haile-Selassie (2001), Senut *et al.* (2001), Vignaud *et al.* (2002), Brunet *et al.* (2002, 2005) and Lebatard *et al.* (2008)
Macaca sylvanus – other macaques	Exponential; offset 5.0; mean 1.0	5.7 (5.0–8.7)	Delson (1980), Jablonski (2002) and Alba *et al.* (2014)
Theropithecus – *Papio*	Normal; mean 5.0; sigma 0.5	5.0 (4.0–6.0)	Leakey (1993) and Frost (2007)
Calibration Set 2			Liedigk *et al.* (2014, 2015)
Cercopithecoidea – Hominoidea	Normal; mean 27.5; sigma 1.8	27.5 (24.0–31.0)	Seiffert *et al.* (2005) and Stevens *et al.* (2013)
Pongo – *Gorilla* / *Pan* / *Homo*	Normal; mean 14.0; sigma 0.5	14.0 (13.0–15.0)	Kelley (2002)
Homo – *Pan*	Normal; mean 6.5; sigma 0.25	6.5 (6.0–7.0)	Haile-Selassie (2001), Senut *et al.* (2001), Brunet *et al.* (2002, 2005), Vignaud *et al.* (2002) and Lebatard *et al.* (2008)
Macaca sylvanus – other macaques	Normal; mean 5.5; sigma 0.5	5.5 (4.5–6.5)	Delson (1980), Jablonski (2002) and Alba *et al.* (2014)
Theropithecus – *Papio*	Normal; mean 5.0; sigma 0.5	5.0 (4.0–6.0)	Frost (2007) and Leakey (1993)

trees using TreeAnnotator 2.3.2 and FigTree 1.4.2 (http://tree.bio.ed.ac.uk/software/figtree/).

RESULTS

We generated complete mtDNA genomes from ten *Chlorocebus* individuals representing all six species of the genus and the nine mtDNA haplogroups detected by Haus *et al.* (2013b). Newly generated mtDNA genomes had lengths of 16 259 to 16 635 bp and consisted of two rRNA genes, 13 protein-coding genes, 22 tRNA genes and the control region.

Contamination of our data set with nuclear mitochondrial pseudogenes (numts) can be excluded, because faecal material contains generally highly degraded nuclear DNA (Thalmann *et al.*, 2004), direct sequencing of PCR products revealed no multiple amplifications of different copies (as indicated by double peaks in the electropherograms), and no inconsistent nucleotides were detected in overlapping sequences. Furthermore, all protein-coding genes were correctly translated without premature stop codons.

For phylogenetic analyses, we generated an alignment including the ten newly generated mtDNA genomes and 53 additional mtDNA genomes of Catarrhini taxa obtained from GenBank. After removing indels and poorly aligned positions, the original alignment of 17 216 bp was reduced to 15 454 bp. ML and Bayesian tree reconstructions resulted in nearly identical tree topologies (Fig. S1) with mainly well-supported branching patterns (ML BS values: >75%, Bayesian PP: 1.0). The only exceptions are the branching patterns among *Theropithecus*, *Lophocebus* and *Papio* [Bayesian tree: *Theropithecus* + *Papio* (PP: 0.85), ML tree: *Lophocebus* + *Papio* (BS: 33%)] and among *Rhinopithecus*, *Pygathrix* and the *Nasalis*/*Simias* clade [Bayesian tree: *Pygathrix* + *Nasalis*/*Simias* (PP: 0.98), ML tree: *Pygathrix* + *Rhinopithecus* (BS: 63%)].

Within *Chlorocebus*, tree reconstructions revealed nine major clades/lineages (Fig. 3) comprising the following species as delineated by phenotypes and geographic regions: C1: *C. tantalus* from Nigeria and Central African Republic and *C. aethiops* from Ethiopia; C2: *C. aethiops* from Ethiopia, but with unknown exact geographic provenance and intermediate phenotype *C. aethiops* × *C. pygerythrus*; C3: *C. cynosuros* from Zambia; C4: *C. cynosuros* with unknown geographic provenance and *C. pygerythrus* from RSA; C5: *C. djamdjamensis* from Ethiopia; C6: *C. pygerythrus* from Ethiopia; C7: *C. sabaeus*; C8: *C. pygerythrus* from Tanzania and C9: *C. tantalus* from Ghana. With exception of *C. sabaeus* and *C. djamdjamensis*, which were both represented by only one sample, phenotypes of all species are found in more than one clade (*C. tantalus*: C1 and C9; *C. aethiops*: C1 and C2; *C. cynosuros*: C3 and C4 and *C. pygerythrus*: C4, C6 and C8), revealing para- and polyphylies within the genus *Chlorocebus*. The branching pattern among lineages and clades is strongly supported in both ML and Bayesian reconstructions, and only the common origin of lineages C3 and C4 (t16) gained lower statistical support (63%, 0.84).

Divergence time estimates obtained from different substitution models, tree priors and calibration sets are highly similar albeit estimates from Calibration Set 1 are slightly older than those from Calibration Set 2 (e.g. Cercopithecoidea – Hominoidea: *c.* 31.9 Myr with Calibration Set 1 vs. 29.5 Myr with Calibration Set 2; Table S2). Considering the high similarity of estimates, hereafter we will discuss only estimated divergence ages derived from the calculation based on the GTR + I + G substitution model, a Yule tree prior and Calibration Set 1. Accordingly, an initial split within *Chlorocebus* occurred ~3.5 Myr [t6; 3.10–3.91 95% highest posterior density (HPD) interval], separating the westernmost species *C. sabaeus* (C7) from all other *Chlorocebus*. A clade comprising *C. djamdjamensis* (C5) and *C. pygerythrus* (C6) from Ethiopia branched off next, ~2.8 Myr (t7; 2.46–3.06 95% HPD), with subsequent divergence of C5 and C6 ~2.1 Myr (t19; 1.83–2.42 95% HPD). As also suggested by lower statistical support values, the next few divergence events (t8, t9, t10 and t19) occurred during a short time period. *C. tantalus* from Ghana (C9) branched off first, ~2.1 Myr (t8; 1.88–2.33 95% HPD). Subsequently, ~2.0 Myr (t9; 1.76–2.17 95% HPD), the remaining lineages divided first into a north-eastern (C1/C2/C8) and a southern (C3/C4) clade. In the north-eastern clade, *C. pygerythrus* from Tanzania (C8) branched off ~1.8 Myr (t10; 1.61–2.00 95% HPD), followed by a subsequent divergence of C1 and C2 ~1.6 Myr (t11; 1.42–1.78 95% HPD). In the southern clade, C3 and C4 separated ~1.8 Myr (t16; 1.64–2.07 95% HPD).

Within *Papio*, *P. ursinus* from South Africa (A) diverged first ~2.5 Myr (t26; 2.23–2.81 95% HPD), followed by a major split between the remaining southern (B/C) and the northern (G/D/F/E) lineages ~2.3 Myr (t27; 2.05–2.55 95% HPD). In the southern clade, *P. kindae* from Zambia (C) branched off ~1.7 Myr (t33; 1.46–1.92 95% HPD) with a subsequent divergence of northern *P. ursinus* and *P. cynocephalus* from Tanzania in Clade B ~0.9 Myr (t34; 0.71–1.02 95% HPD). The northern lineages initially divided into a north-western (D/F/E) and a north-eastern (G) clade ~1.8 Myr (t28; 1.55–1.97 95% HPD). Within the latter clade, *P. cynocephalus* from Tanzania split off ~0.4 Myr (t29; 0.33–0.50 95% HPD), with subsequent divergence of eastern *P. anubis* and *P. hamadryas* from Eritrea ~0.2 Myr (t30; 0.17–0.29 95% HPD). The three

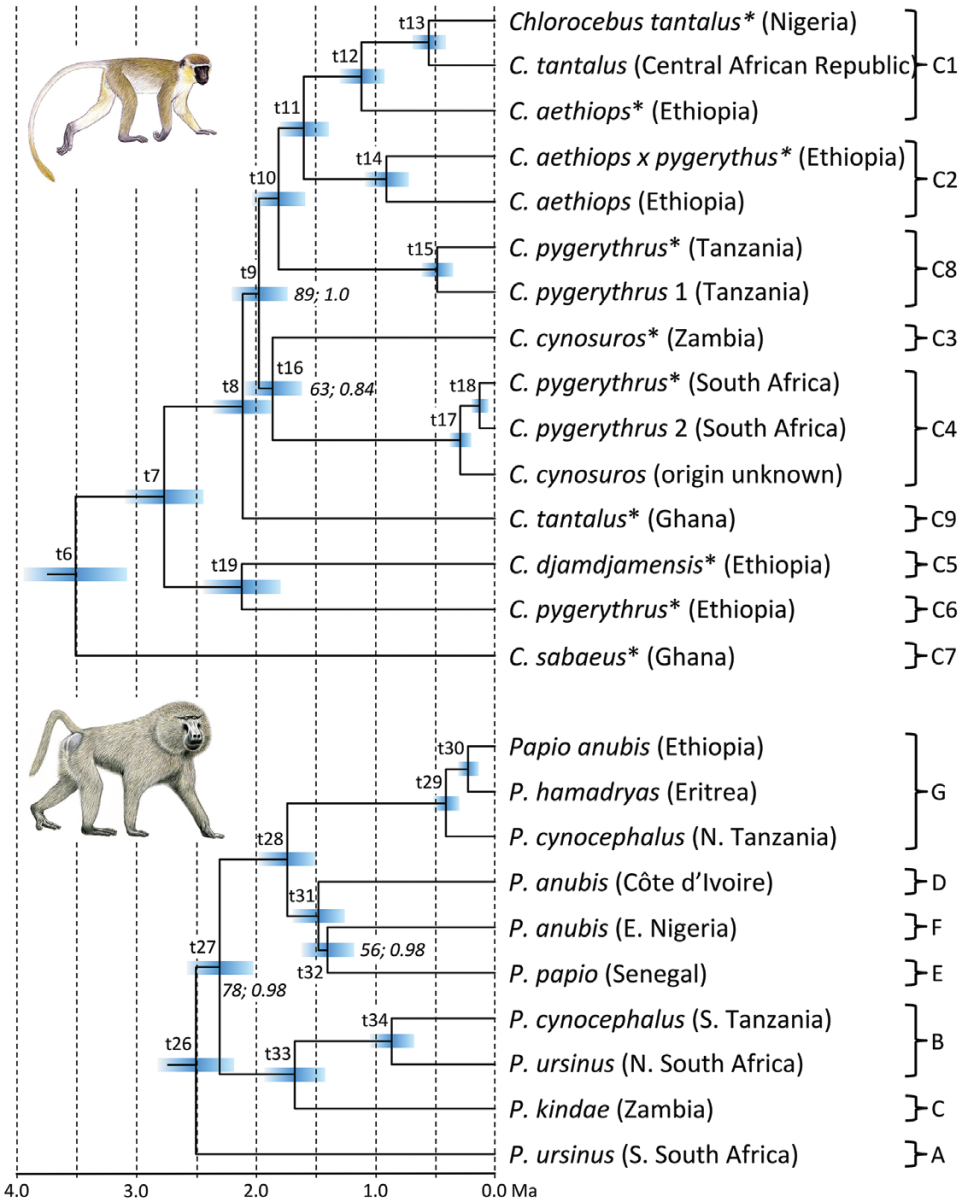

Figure 3. Ultrametric subtrees showing phylogenetic relationships and divergence times within *Chlorocebus* (above) and *Papio* (below) as obtained from the BEAST reconstruction using the GTR + I + G substitution model, a Yule tree prior and Calibration Set 1 (the complete tree is shown in Fig. S1). Newly generated *Chlorocebus* mtDNA genome sequences are marked with an asterisk (see Table S1). C1–C9 indicate the nine major mtDNA clades within *Chlorocebus* according to Haus *et al.* (2013b); x = putative hybrid due to intermediate phenotype. A–G indicate the seven major mtDNA clades within *Papio* (Zinner et al., 2009, 2013). All nodes are numbered (*Chlorocebus*: t6–t18; *Papio*: t26–t34). BS and PP values lower than 100% and 1.0 are given at respective nodes. Blue bars represent 95% confidence intervals of divergence times. The timescale is calibrated in million years. The estimated divergence times are given in Table S2. Primate illustrations by Stephen Nash.

lineages within the north-western clade diverged ~1.5 Myr (t31; 1.28–1.67 95% HPD) and ~1.4 Myr (t32; 1.21–1.60 95% HPD), respectively, although resolution of the branching pattern among them is relatively low (BS: 56%, PP: 0.98).

DISCUSSION

We reconstructed a well-supported mtDNA phylogeny for *Chlorocebus* and estimated divergence times of the different lineages using complete mtDNA genome data.

Further, based on our phylogenetic reconstruction, we propose a phylogeographic scenario for *Chlorocebus* and compared it with a scenario for *Papio*.

As in Haus *et al.* (2013b), the mtDNA clades revealed from complete mtDNA genomes reflect geographic distributions rather than nominal species. All clades demonstrate good correspondence to geographic regions, except for one *C. aethiops* sample from Ethiopia, which clusters with *C. tantalus* from Nigeria and Central African Republic into C1. This exception, however, is in concordance with findings by Shimada (2000) and Haus *et al.* (2013b). Para- and polyphyletic relationships for all *Chlorocebus* species were confirmed, except for *C. sabaeus* and *C. djamdjamensis*. However, these two taxa were represented by only one sample each, so that possible nonmonophyletic relationships could not be detected. Likewise, also the nuclear genomic phylogeny presented by Warren *et al.* (2015) is based on a single specimen per species and, thus, allows no inferences about possible para- or polyphylies. Since, in our phylogenetic reconstruction, geographically close lineages cluster together, we follow the suggestion by Haus *et al.* (2013b) that introgression is the most possible cause for the presence of para- and polyphylies, although other factors such as incomplete sorting cannot be excluded.

Most splits in our phylogenetic reconstruction have stronger support than in Haus *et al.* (2013b), and the branching pattern among *Chlorocebus* lineages/clades is almost completely resolved; only node t16 gains lower statistical support. Although clades C1–C9 correspond to the nine clades distinguished by Haus *et al.* (2013b), the order of branching in our tree reconstruction is different. The first divergence within *Chlorocebus* is between the westernmost *C. sabaeus* clade (C7) and all other lineages. This is in agreement with other mtDNA and nuclear DNA studies (Wertheim & Worobey, 2007; Chatterjee *et al.*, 2009; Guschanski *et al.*, 2013; Ayouba *et al.*, 2015; Warren *et al.*, 2015). The most recently emerged lineage within *Chlorocebus* is represented by the southern lineage consisting of *C. cynosuros* and *C. pygerythrus*. This also is in agreement with previous studies on mtDNA (Guschanski *et al.*, 2013; Ayouba *et al.*, 2015) and nuclear DNA (Warren *et al.*, 2015), but in contrast to Haus *et al.* (2013b), whose analysis could not resolve the relationships of the southern lineages with other clades. Within the southern lineage, as in Haus *et al.* (2013b), *C. cynosuros* is found in two clades: C3 and C4, although neither Haus *et al.* (2013b) nor our analysis provided significant statistical support for this splitting. As suggested by Haus *et al.* (2013b), this pattern can simply be due to wrong taxonomic determination of the respective specimens. Unfortunately, since the geographic provenance of the *C. cynosuros* sample from C4 is unknown, this question cannot be solved here.

The overall catarrhine divergence date estimates are on a similar timescale as estimates from earlier molecular studies (Chan *et al.*, 2010; Perelman *et al.*, 2011; Roos *et al.*, 2011; Liedigk *et al.*, 2012). Within *Chlorocebus*, our dates appear older than the estimates obtained by Guschanski *et al.* (2013), who dated the radiation within *Chlorocebus* to 2.2–1 Myr. Interestingly, all mtDNA-based estimates for the *Chlorocebus* radiation are in stark contrast to those based on nuclear genomic data. According to Warren *et al.* (2015), the radiation within *Chlorocebus* is much younger and occurred within the last 550 000 years. This discrepancy could be due to different population histories reflected by different parts of the genome or more likely due to differences in applied methods for estimating divergence times [fossil-based calibration in this study, substitution rate in Warren *et al.* (2015)]. Within *Papio*, our mtDNA-based estimates are generally consistent with dates from Zinner *et al.* (2013), who dated the radiation within the genus to 2.2–1.0 Myr.

Phylogeographic implications

The first split sets the westernmost clade representing *C. sabaeus* as sister taxon to all other *Chlorocebus* taxa. This suggests the origin of *Chlorocebus* to be north of the Central African rainforest. Our phylogenetic reconstruction further suggests a complicated biogeographic history with at least two temporally independent range expansions into eastern Africa [C5 and C6 (Ethiopia) and C1, C2 and C8 (East Africa)] and one into southern Africa (C3 and C4). Interestingly, similar mitochondrial haplotypes are found in *C. tantalus* from Nigeria/Central African Republic and *C. aethiops* from Ethiopia (C1a and C1b). Both taxa are parapatric, but a contact zone might have existed or still exists in southern Sudan.

Although the divergence of *Papio* appears to be slightly more recent than the divergence of *Chlorocebus*, it occurred on a similar timescale (2.5–1.4 Myr and 3.5–1.6 Myr, respectively). According to the phylogenetic scenario suggested by Zinner *et al.* (2009, 2011b, 2013), *Papio* originated in southern Africa and was first prevented from dispersal to the north by the equatorial forest belt. Due to climate changes during glacial periods, the savannah biome expanded, enabling the dispersal to the north. After reaching the northern part of the range, baboons further dispersed to the west and to the east, possibly in two subsequent waves. The same two-wave pattern was suggested for giraffes *Giraffa camelopardalis*, as populations from Niger seem to be more closely related to East African than to neighbouring Central African populations

(Hassanin *et al.*, 2007). A scenario of several temporally independent expansions is also conceivable for *Chlorocebus*.

The para- and polyphyletic relationships within *Chlorocebus* and *Papio* suggest a complicated biogeographic history of these genera. Although it is difficult to make inferences on temporal concordance of evolutionary and climatic events, the last 10 Myr were characterized by repeated climate fluctuations in Africa, leading to dynamic changes in forest and savannah cover (Bonnefille, 2010). The dispersing lineages within both *Chlorocebus* and *Papio* probably experienced multiple phases of isolation and reconnection, triggered by recurrent expansion of unsuitable forest or desert habitats during Pleistocene (2.588–0.012 Myr) glacial and interglacial periods, creating various isolated savannah refuges at certain periods which were subsequently reconnected (Nichol, 1999; deMenocal, 2004). The speciation within both *Chlorocebus* and *Papio* also appears to be temporally in accordance with general aridification in Africa around 2.9–2.4 Myr that led to changes in savannah habitats: savannah expansions in West and East Africa and loss of savannah habitat in North Africa (deMenocal, 2004; Bonnefille, 2010).

The phylogeographic scenarios for *Chlorocebus* and *Papio* represent two main phylogeographic patterns of sub-Saharan African savannah mammals. *Chlorocebus* represents a scenario with an initial split of western and eastern clades with subsequent dispersal to the south accompanied by divergence into north-eastern and southern clades. A similar phylogeographic pattern of an initial west–east division has been suggested for other African savannah animals, such as common warthogs *Phacochoerus africanus* (Muwanika *et al.*, 2003), African elephants *Loxodonta africana* (Nyakaana, Arctander & Siegismund, 2002), African buffalo *Syncerus caffer* (Van Hooft, Groen & Prins, 2002) and roan antelopes *Hippotragus equinus* (Alpers *et al.*, 2004). *Papio*, on the other hand, represents the second phylogeographic pattern with an initial separation of southern and northern lineages with subsequent splits into eastern and western clades in the North. Apart from baboons, this scenario was shown for antelopes of the genus *Alcelaphus* (Arctander, Johansen & Coutellec-Vreto, 1999; Flagstad *et al.*, 2001), giraffes *G. camelopardalis* (Hassanin *et al.*, 2007) and lions *Panthera leo* (Bertola *et al.*, 2011).

CONCLUSION

We generated a phylogeny for the genus *Chlorocebus* based on complete mtDNA genomes of ten samples representing all species and all major mtDNA clades. We obtained stronger statistical support in the phylogenetic reconstruction than in previous studies based on partial mtDNA sequence data. In accordance with Haus *et al.* (2013b), the nine major mtDNA clades indicate several cases of paraphyly within the genus and reflect geographic distributions rather than taxonomy. Our results suggest an initial west–east division in the northern part of the genus' range with subsequent divergence into a north-eastern and a southern clade. The general phylogeographic scenario for the dispersal of *Chlorocebus* thus contrasts with that for another widespread African savannah primate genus, baboons of the genus *Papio*, which diverged on a similar timescale. The origin of baboons is believed to have been in southern Africa with an initial south–north division with subsequent separation of eastern and western clades in the northern part of the range. The opposing dispersal scenarios for *Chlorocebus* and *Papio* thus represent primate cases of the two main phylogeographic dispersal patterns found in sub-Saharan African savannah mammals.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article at the publisher's website:

Table S1. Samples, their origin and GenBank accession numbers.
Table S2. Support values and estimated divergence ages.
Figure S1. Ultrametric tree showing phylogenetic relationships and divergence ages among 63 mtDNA genome sequences as obtained from a BEAST reconstruction using the GTR + I + G substitution model, a Yule prior and calibration set 1. Blue bars indicate 95% credibility intervals of divergence times and the timescale below shows million years before present. Nodes are numbered and refer those listed in Figure 3 and Table S2. Details about ML bootstrap support and Bayesian posterior probabilities as well as divergence times with their 95% credibility intervals are provided in Table S2.