Genetic diversity in the lion (panthera leo (Linnaeus 1758)) : unravelling the past and prospects for the future

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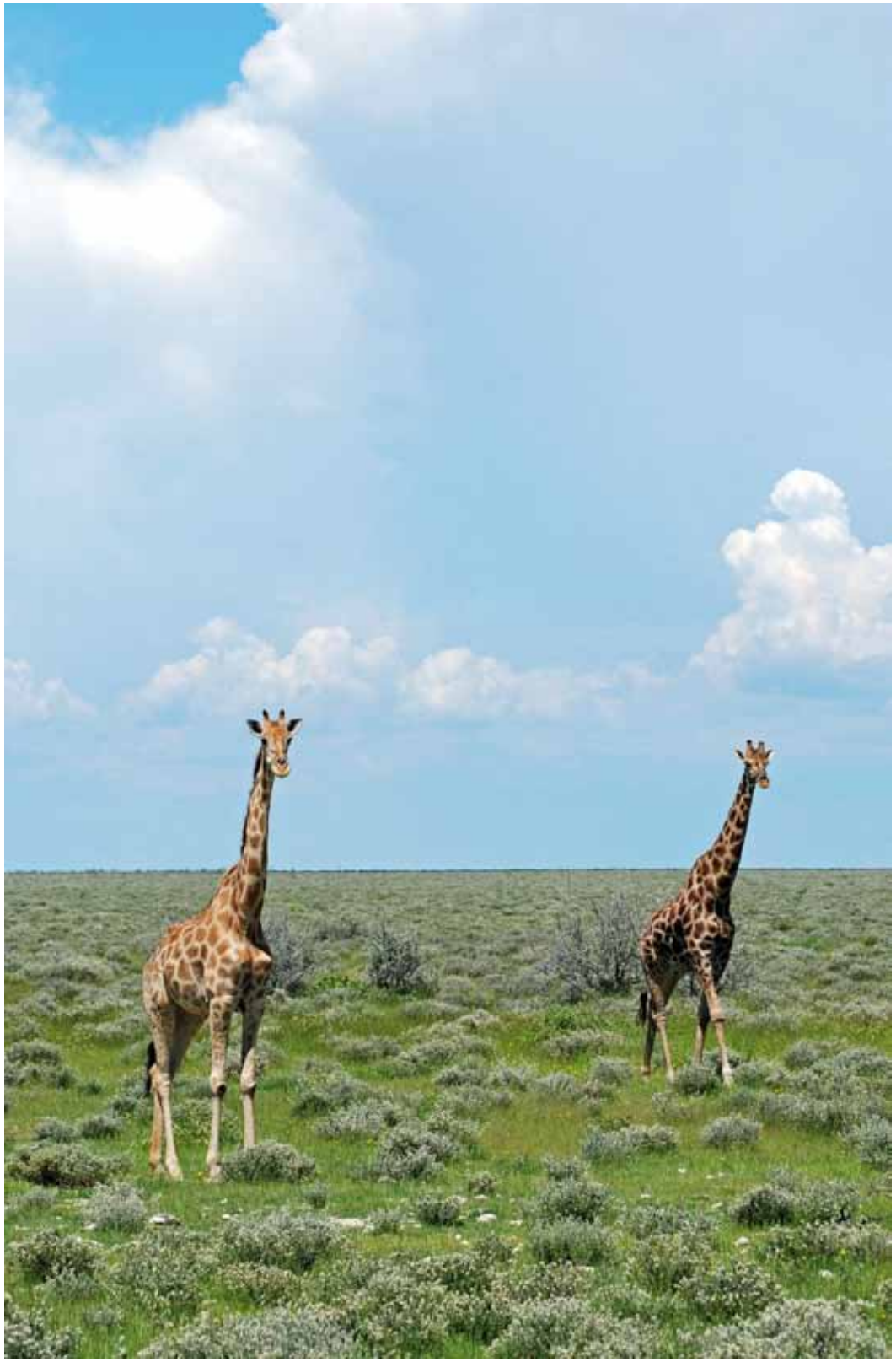

4 Chapter

Phylogeographic patterns in Africa and high resolution delineation of genetic clades in the African lion

(under review)

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Abstract

Numerous African savannah mammals show a congruent phylogenetic pattern in which populations in West/Central Africa are distinct from populations in East/Southern Africa. However, for the lion, all African populations are currently classified as a single subspecies (*Panthera leo leo*), while the only remaining lion population in Asia is considered to be distinct (*Panthera leo persica*). In this study, we assess the phylogeography of the lion, by analysing mitochondrial DNA data of populations throughout the complete geographic range of the lion. This reveals six supported clades and a strongly supported ancestral dichotomy with populations from the northern part of the range (West Africa, Central Africa, North Africa/Asia) on one branch, and populations from the southern part of the range (North East Africa, East/Southern Africa and South West Africa) on the other. This phylogeography is congruent with patterns found in other savannah mammals and is addressed in relation to large scale environmental changes in Africa, driven by climate. The degree of divergence and the nested position of the Asiatic subspecies strongly support the revision of current lion taxonomy, and we propose to recognize a northern and a southern subspecies as this is more in line with the evolutionary history of the lion.

Keywords: phylogeography, Africa, lion (*Panthera leo*), mitochondrial genome, African climate history, savannah mammals

Introduction

Insight into the genetic lineages in a species is of importance, because phylogeographies contribute to the understanding of evolutionary histories as well as to the design of effective conservation strategies. On the African continent we observe strongly congruent phylogenetic patterns for savannah mammal species with a comparable, continent-wide distribution. The distribution of subspecies, and species within species complexes, tends to follow a north-south axis in sub-Saharan Africa, in which West and Central Africa is inhabited by other taxonomic groups than East and Southern Africa (regions defined following the lion conservation strategies (IUCN SSC Cat Specialist Group 2006a; b)) (Table 1 and Figure 1). This north-south dichotomy was further confirmed by genetic data from primates, elephant, rhinoceros, numerous other ungulates and carnivores (see Table 1 for accompanying references). For most species mentioned distinct subspecies are recognized, but not for the African lion (*Panthera leo leo*), which is reflected in the conservation strategy of the species (Bauer *et al.* 2012).

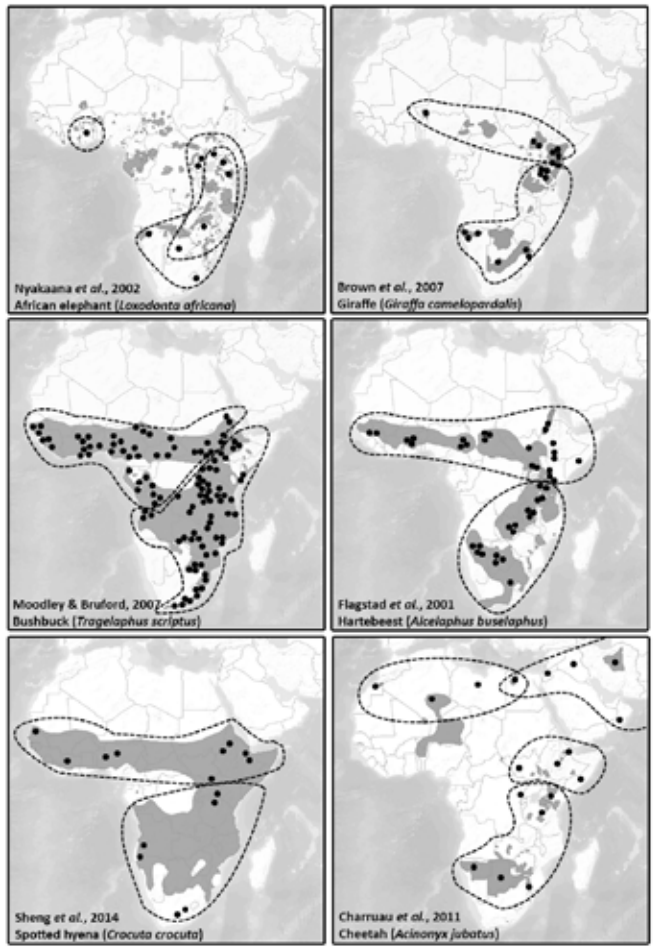


Figure 1. Examples from six species for which a dichotomy between West/Central African populations and populations in East/Southern Africa has been shown in phylogenetic data: African elephant (*Loxodonta africana*), giraffe (*Giraffa camelopardalis*), bushbuck (*Tragelaphus scriptus*), hartebeest (*Alcelaphus buselaphus*), spotted hyena (*Crocuta crocuta*) and cheetah (*Acinonyx jubatus*). Sample locations (black dots) are indications and are not necessarily proportional to the number of collected samples. The most basal phylogenetic groups identified are delineated. Range data from IUCN (IUCN 2014).

Table 1. Overview of African mammals for which a distinction between West/Central African populations and populations in East/Southern Africa has been described (Kingdon 2007; IUCN 2014).

Taxonomic distinction between West/Central Africa and East/Southern Africa				
Order	**Species (complex)**	**(sub)Species**	**Phylogeography references***	**Genetic marker**
Primates	Babboon complex (*Papio)*	5 species	Zinner et al. (2009)	mtDNA
	Green monkey complex (*Chlorocebus*)	6 species	Haus et al. (2013)	mtDNA
	Senegal galago (*Galago senegalensis*)	4 subspecies	-	-
Hyracoidea	Rock hyrax complex (*Procavia*)	5 species	-	-
Perissodactyla	Black rhino (*Diceros bicornis*)	4 subspecies	Harley et al. (2005)	msats
	White rhino (*Ceratotherium simum*)	2 subspecies	-	-
Artiodactyla	Giraffe (*Giraffa camelopardalis*)	9 subspecies	Brown et al. (2007); Hassanin et al. (2007); Bock et al. (2014)	mtDNA + msats
	African buffalo (*Syncerus caffer*)	3-4 subspecies	Van Hooft et al. (2002); Smitz et al. (2013)	mtDNA + Y chromosomal msats
	Bushbuck (*Tragelaphus scriptus*)	2 groups, numerous subspecies	Moodley & Bruford (2007)	mtDNA
	Greater kudu (*Tragelaphus strepsiceros*)	3 subspecies	-	-
	Eland complex (*Tragelaphys debianus / T. oryx*)	2 species	-	-
	Bush duiker (*Sylvicapra grimmia*)	8 groups, numerous subspecies	-	-
	Dwarf antelope complex *Neotragus*	3 species	-	-
	Oribi (*Ourebia ourebi*)	7-13 subspecies	-	-
	Reedbuck complex (*Redunca redunca / R. arundinum*)	2 species	-	-
	Mountain reedbuck (*Redunca fulvorufula*)	3 subspecies	-	-
	Kob / Puku complex (*Kobus kob / K. vardoni*)	2 species	Lorenzen et al. (2007)	mtDNA + msats
	Lechwe complex (*Kobus leche / K.megaceros*)	2 species	-	-
	Waterbuck (*Kobus ellipsiprymnus*)	2 subspecies	Lorenzen et al. (2006)	mtDNA + msats
	Red-fronted gazelle (*Eudorcas rufifrons*)	5 subspecies	-	-
	Grant's gazelle complex (*Nanger*)	3 species	-	-
	Topi (*Damaliscus lunatus*)	5-6 subspecies	-	-
	Hartebeest (*Alcelaphus buselaphusi*)	8 subspecies	Arctander et al. (1999); Flagstad et al. (2001)	mtDNA
	Roan antelope (*Hippotragus equinus*)	2 groups, 6 subspecies	Alpers et al. (2004); Matthee & Robinson (1999)	mtDNA + msats
	Oryx complex (*Oryx*)	3 species	-	-
Carnivora	Egyptian mongoose (*Herpestes ichneumon*)	up to 11 subspecies	Gaubert et al. (2011)	mtDNA
	Slender mongoose (*Herpestes sanguineus*)	up to 50 subspecies	-	-
	White-tailed mongoose (*Ichneumia albicauda*)	6 subspecies	Dehghani et al. (2008)	mtDNA
	Common genet (Genetta genetta)	3 groups, numerous subspecies	Gaubert et al. (2011); Delibes & Gaubert (unpub.)	mtDNA
	African civet (*Civettictis civetta*)	5 subspecies	-	-
	Wild cat (*Felis silvestris*)	5 subspecies	Driscoll et al. (2007)	mtDNA + msats
	Caracal (*Caracal caracal*)	8 subspecies	-	-
	Cheetah (*Acinonyx jubatusi*)	5 infra-specific taxa assessed	Freeman et al. (2001); Charruau et al. (2011)	mtDNA + msats
No taxonomic distinction				
Order	**Species (complex)**	**(sub)Species**	**Phylogeography references***	**Genetic marker**
Proboscidea	African (bush) elephant (*Loxodonta africana*)	-	Nyakaana et al. (2002)	mtDNA + msats
Pholidota	Ground pangolin (*Manis temmicnkii)*	-	-	-
Tubulidentata	Aardvark (*Orycteropus afer*)	-	-	-
Artiodactyla	Common warthog (*Phacochoerus africanus*)	-	Muwanika et al. (2003)	mtDNA
Carnivora	African wild dog (*Lycaon pictus*)	-	-	-
	Zorilla (*Ictonyx striatus*)	-	-	-
	Honey badger (*Mellivora capensis*)	-	-	-
	Banded mongoose (*Mungos mungo*)	-	-	-
	Marsh mongoose (*Atilax paludinosus*)	-	-	-
	Spotted hyena (*Crocuta crocuta*)	-	Rohland et al. (2005), Sheng et al. (2014)	mtDNA
	Serval (*Leptailurus serval*)	-	-	-
	African Leopard (*Panthera pardus pardus*)	(one subspecies in Africa)	-	-
	African Lion (*Panthera leo leo*)	(one subspecies in Africa)	Dubach et al. (2013); Barnett et al. (2014); Bertola et al. (2011); Bertola et al. (submitted)	mtDNA + msats

* Only references that cover the complete (sub)species's range on the African continent are listed. Publications focussing on a more regional level were excluded.

Phylogenetic data of lion populations indicate that current taxonomy does not sufficiently reflect the genetic diversity within the African lion (Dubach *et al.* 2005, 2013; Barnett *et al.* 2006a; b, 2014; Antunes *et al.* 2008; Bertola *et al.* 2011; Bruche *et al.* 2012). Notably, lion populations from West and Central Africa have a distinct phylogenetic position, with a nested position for the Asiatic subspecies (*Panthera leo persica*) (Barnett *et al.* 2006a; b, 2014; Bertola *et al.* 2011). The validity of the subspecies status of the Asiatic lion, nowadays confined to a single population in India, is thereby challenged. However, previous studies describing distinct genetic lineages within the African lion did not thoroughly cover the West and Central African region and based there results on relatively small sample sizes (Dubach *et al.* 2005, 2013; Barnett *et al.* 2006a; b, 2014; Bertola *et al.* 2011). The position of the these populations and their relation to the Asiatic subspecies in the phylogenetic tree remained largely unresolved (Barnett *et al.* 2006b, 2014; Bertola *et al.* 2011).

The present study aims to provide a more complete overview of genetic diversity within the African lion and compares this to phylogeographic patterns and taxonomy in a range of African savannah mammals. In addition, we estimate the dates of the major splits in the phylogenetic tree and aim to relate observed patterns to the dynamic climate history of Africa. Previous studies have shown that mitochondrial DNA (mtDNA) loci produce phylogenies that are not contradictory with phylogenies based on autosomal data (Antunes *et al.* 2008; Bertola *et al.* submitted), which indicates that mtDNA is an appropriate marker for making this case. For sixteen lions the complete mitochondrial genome was analyzed. In addition, 1454 base pairs (bp) of the mtDNA were analyzed for 178 lions throughout their complete geographic range (see Figure 2 and Supplemental Table S1 for sampling locations), including samples from each of the Lion Conservation Unit (LCU) in West and Central Africa that still contains a recently confirmed resident lion populations (Riggio *et al.* 2012; Henschel *et al.* 2014). To reconstruct the evolutionary history of the West and Central African lion, museum samples from extinct populations in North Africa and Asia, representing a historical connection between the African and the Asiatic subspecies, were obtained and an ancient DNA (aDNA) approach was used for processing. These museum samples also included lions from areas from which it was not possible to include modern samples of wild lions.

This is the first study in which a number of approaches, including Next Generation Sequencing (NGS) techniques, are applied on a dataset of 194 lions from 22 different countries throughout the complete geographic range and most of the historic range of the modern lion. These data contribute to a better understanding of evolutionary forces that shaped the phylogenetic patterns observed among numerous savannah mammals on the African continent. Results should be translated into recommendations for the management of diverse species and populations. In particular, we challenge current lion taxonomy that recognizes only the African and the Asiatic subspecies, and we investigate options for a revision that is more parsimonious with the recently improved understanding of the evolutionary history of the lion.

Materials and Methods

In total, 194 samples from lions of 22 different countries were analyzed, including samples previously described in Bertola *et al.* (2011), Barnett *et al.* (2014) and Bertola *et al.* (submitted) (Supplemental Table S1 and Figure 2 for samples locations). Blood, tissue or scat samples were collected from free-ranging individuals or captive lions with proper documentation of their breeding history. A total of 16 museum specimens, collection dates ranging from 1831 to 1967, was added to the dataset. Maxilloturbinal bone was sampled, unless another sample was more readily available. Samples were collected in full compliance with specific legally required permits (CITES and permits related to national legislation in the countries of origin). For details on sample storage and processing, see Supplemental Information S1, Supplemental Tables S2 and S3.

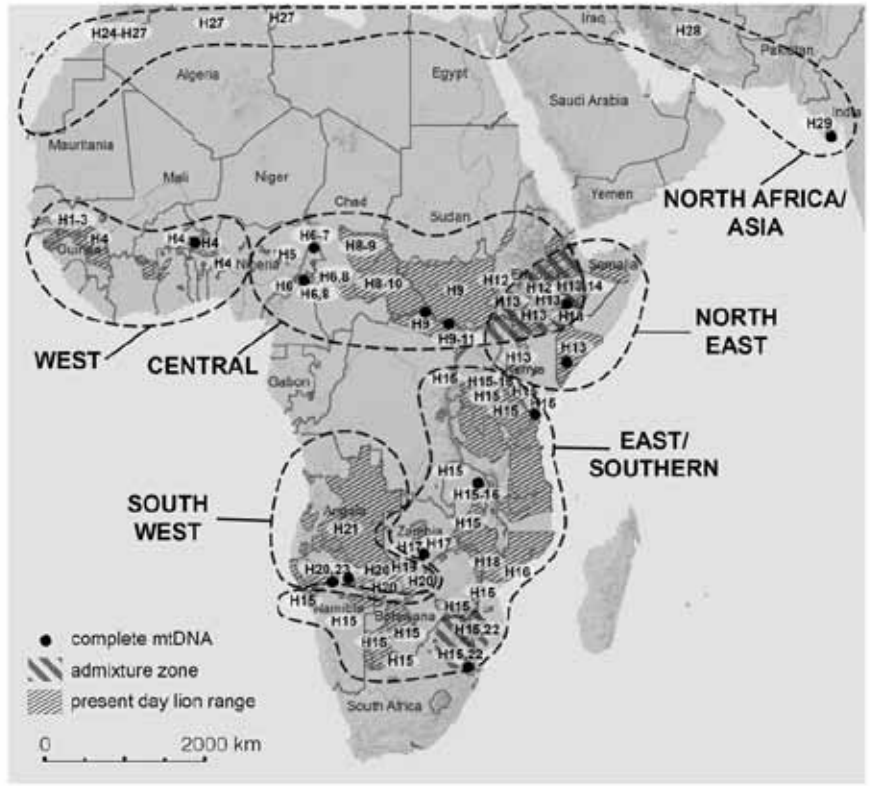


Figure 2. Locations of lion samples and haplotype numbers included in this study. Proposed phylogenetic lineages are delineated. Admixture zones in which haplotypes from different phylogenetic lineages are found are indicated by shading. Lion range data from IUCN (2014).

For all available samples, analyses were performed on alignments consisting of cytochrome b, tRNAThr, tRNAPro and the left domain of the control region (hereafter referred to as cytB+ctrl reg.) (1454 bp, 202 sequences), the complete mitogenome (16756 bp, excluding RS-2 and RS-3, 23 sequences) and an alignment including all sequence data, where ambiguous nucleotides were added to create sequences of equal length. Bayesian analysis was performed using MrBayes v.3.1.2

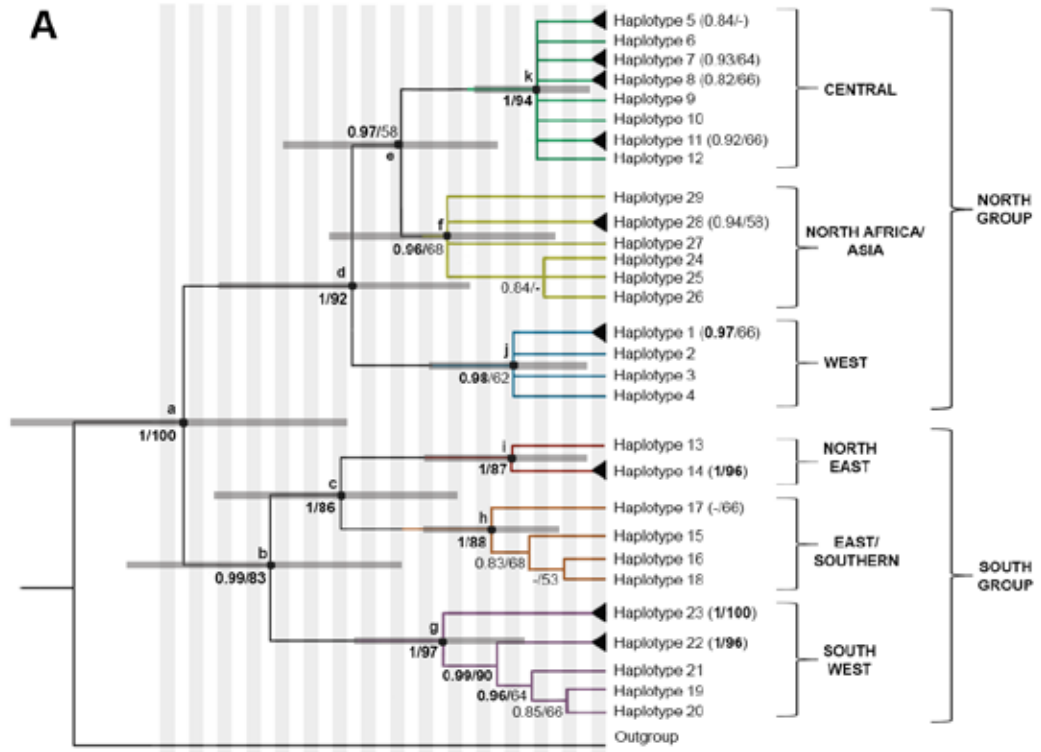
(Huelsenbeck & Ronquist 2001; Ronquist *et al.* 2012), using parameters determined by MrModeltest2 (v.2.3) (Nylander 2004) and Maximum Likelihood (ML) analysis was done in Garli (Zwickl 2006). Branches receiving >0.95 PP in Bayesian analysis and/or 70 bootstrap support in ML analysis are considered to be significantly supported. A haplotype network was created using the median-joining algorithm in Network 4.6.1.1 (available from www.fluxus-engineering.com) with equal weighing of all characters.

BEAST v.1.7.5 (Drummond & Rambaut 2007) was used to obtain estimated values for the time to most recent common ancestor (TMRCA) to date splits in the lion tree. Five independent runs of 50 million iterations were performed, discarding the first ten percent of each run as burnin, and using the same model as was used for Bayesian analysis and relaxed molecular clock setting. Fossil evidence for the origin of *Panthera*, the *P.leo-P.pardus* group and *P.leo* (including *P. leo spelaea*) was used for calibration and set to 3.8 million years before present (Ma), 1.6 Ma and 0.55 Ma respectively (Kurten & Anderson 1980; Janczewski *et al.* 1995; Burger *et al.* 2004; Johnson *et al.* 2006; Davis *et al.* 2010). Convergence of the runs was assessed in Tracer. Logcombiner, Treeannotator and Figtree (available from http://tree.bio.ed.ac.uk/software/figtree/) were used to visualize the results.

Results

Bayesian and ML trees were constructed from three different alignments: 1) cytB+ctrl reg., 2) the complete mitogenome, and 3) a combination of both datasets. All showed identical topology and trees including the complete mtDNA showed strongly significant support for a basal split separating lions in the northern part of their range (North group: West Africa, Central Africa, and North Africa/ Asia) and lions in the southern part of their range (South group: North East, East/Southern, and South West Africa) (Figure 3A). Within the North group, a clade that included all Asiatic lions and aDNA sequences from North Africa and Iran was significantly supported, as was a clade with Central African lions and the clade with West African lions. Lions from Central Africa and the North Africa/ Asia clade are grouped together on a well-supported branch. In the South group, three major groups can be distinguished: a South West group, an East/Southern group and a North East group. All three clades are significantly supported, as is the branch combining the East/Southern and North East group. The same structure can be seen in the haplotype network based on cytB+ctrl reg. (Figure 4). The observed groups are indicated together with the sample location in Figure 2. Only in two cases did we observe haplotypes from distinct phylogenetic groups in one geographic region: in Ethiopia we found haplotypes from the Central Africa group as well as from the North East group, and in the Republic of South Africa (RSA) we found haplotypes from East/Southern and the South West group (Figure 2, shaded areas).

The most recent common ancestor of all modern lions was estimated to have existed around 291.7 thousand years ago (291.7 ka) (95% Highest Posterior Density (HPD): 178.0-417.7 ka). The split of the South group is older than the North group, estimated to be 231.3 ka (95% HPD: 132.3-338.7 ka) and 174.7 ka (95% HPD: 94.9-276.7 ka), respectively. For all major clades the date of the most recent common ancestor was estimated and compared to results from previous publications (Supplemental Table S4, Figure 3A+B).



B

Clade	Node	Date (95% HPD)	Clade	Node	Date (95% HPD)	Clade	Node	Date (95% HPD)	Clade	Node	Date (95% HPD)
Modern Lion	a	291.7 ka (178.0-417.7)	North	d	174.7 ka (94.9-276.7)	South West Africa	g	113.8 ka (55.2-189.0)	West Africa	j	63.4 ka (15.1-129.1)
South	b	231.3 ka (132.3-338.7)	Central Africa - India	e	141.1 ka (71.7 - 216.6)	East/ Southern Africa	h	78.1 ka (37.2-132.1)	Central Africa	k	49.6 ka (20.7-91.0)
East/Southern - North East Africa	c	183.7 ka (107.0-271.1)	India - North Africa	f	110.8 ka (39.7-200.1)	North East Africa	i	63.9 ka (18.0-118.6)			

Figure 3. Phylogenetic analyses for the complete lion dataset, including sixteen mitochondrial genomes and 175 cytb+ctrl reg. sequences. A: Phylogenetic tree of lion populations throughout their complete geographic range, based on complete mitochondrial genomes and cytB+ctrl reg. sequences. Branch colours correspond to haplotype colours in Figure 4. Support is indicated as posterior probability (Bayesian analysis)/bootstrap support (ML analysis). Branches with a single haplotype have been collapsed to improve readability. Support for these branches is indicated by a black triangle at the tip of the branch (support shown in the label). Nodes which have been included for divergence time estimates are indicated with letters and 95% HPD node bars. Distance to outgroup and nodes without dated split is not in proportion to divergence time. B: divergence estimates and 95% HPD from BEAST analysis, also indicated as error bars in Figure 3A.

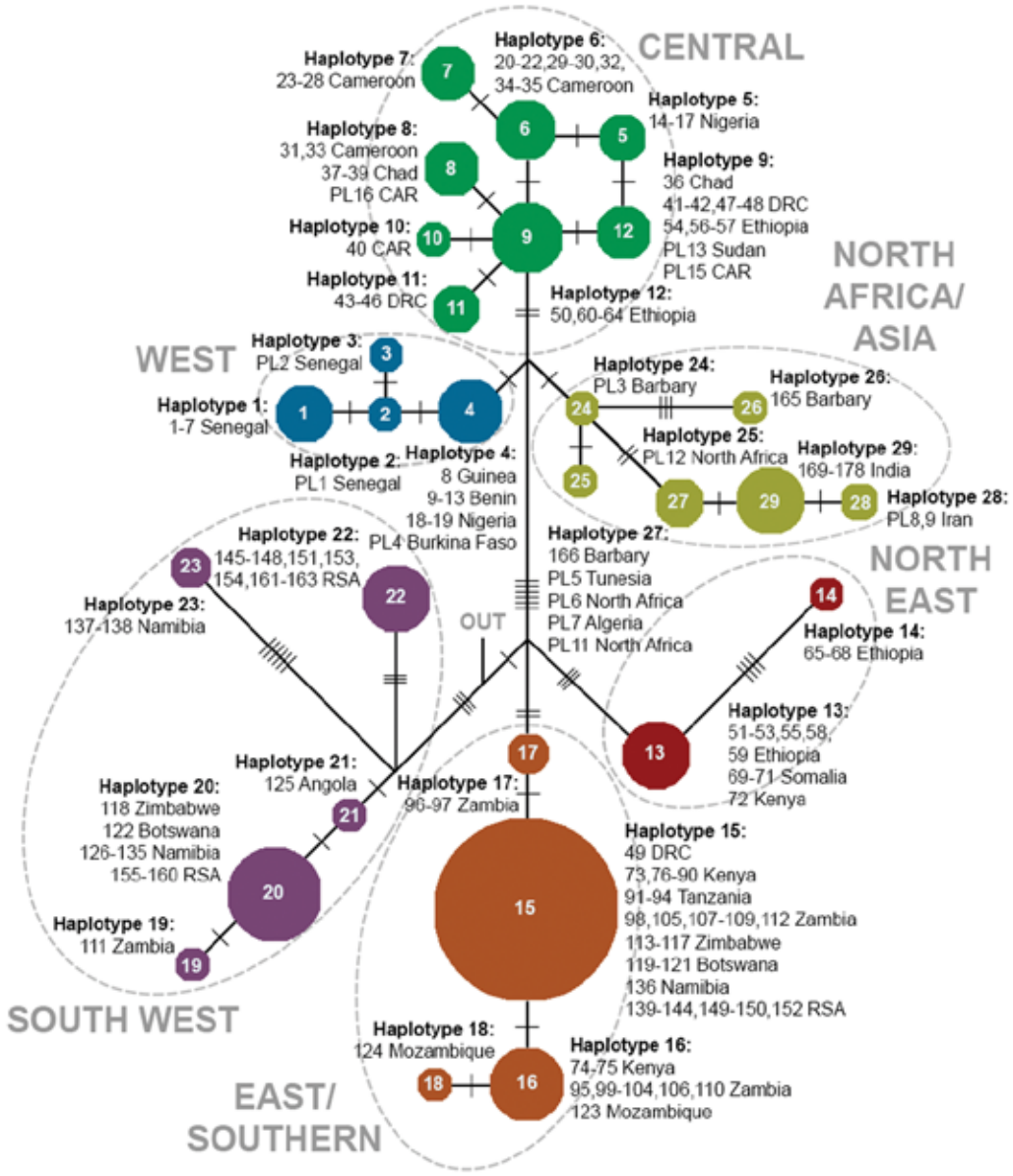


Figure 4. Haplotype network based on cytB+ctrl reg. sequences of lions throughout their entire geographic range. Dashed lines indicate the groups discerned by Bayesian/ML analysis in Figure 3. Haplotype size is proportional to its frequency in the dataset. Hatch marks represent a change in the DNA sequence. The connection to outgroup species is indicated by "OUT".

Discussion

In this paper, we describe the phylogenetic relationships of lion populations throughout their entire geographic range based on 194 sequences of cytB+ctrl reg., including 30 aDNA sequences, and 16 sequences of the complete mitogenome. This has led to strong support for a basal dichotomy between lion populations from the northern part of their range and those from the southern part. Six major phylogenetic groups are identified: West Africa, Central Africa and North Africa/Asia (North group) and North East, East/Southern and South West (South group).

This study included samples from 22 lion range states, including all LCUs with a confirmed lion population in West and Central Africa, and including extinct populations, covering a major part of the historical geographic range of the modern lion. Our results show that lion populations that were previously described as unique, as was the case for the Addis Ababa lions (Bruche *et al.* 2012) and for the Sabi Sands lions (Dubach *et al.* 2005) are most likely the result of incomplete sampling. Angola is represented by one aDNA sample only, which clusters to the South West group. Although it is difficult to draw conclusions for the entire Angolan lion population, this suggests that the captive Angolan lions that were included in previous phylogenetic studies (Antunes *et al.* 2008; Bertola *et al.* 2011) are not pure-bred Angolan. Pedigree information also shows that there is no complete documentation of the female lineage in this captive population (Steinmetz *et al.* 2006). Samples from zoos and museums were only included in our study when decisive information was available on the origin of the individual or its free-ranging ancestors (for additional information see Supplemental Information S2).

Based on the available datapoints, a proposed range of the haplogroups is shown in Figure 2. Two areas of admixture between distinct lineages are indicated by shading. Although the Rift Valley has been proposed as a barrier for gene flow in lions (Burger *et al.* 2004; Dubach *et al.* 2005; Barnett *et al.* 2006a; b, 2009; Bertola *et al.* 2011), our denser sampling of the connecting region between the North and South groups shows that it does not completely prevent a mixture of haplotypes from the two basal branches in the phylogenetic tree (haplotype 9, 12-14). The second admixture zone is located around Kruger National Park (NP) and Limpopo-Venetia National Reserve (NR), RSA, in which we detect haplotypes from the South West group (haplotype 20 and 22) in addition to haplotypes from the East/Southern group (haplotype 15). Since lions from other parts of RSA and the Southern range of Botswana and Namibia also cluster to the East/Southern group, it is likely that the mixture of haplotypes in the Kruger/Limpopo area is the result of human-induced translocations. Lions from Etosha have frequently been used in translocations and it is known that private reserves adjacent to Kruger NP, that were initially fenced off, are now connected to the park (Miller *et al.* 2013).

The pattern we describe for the lion is highly congruent with phylogeographic data from different taxonomic groups on a range of trophic levels, indicating environmentally driven evolution. Several phylogeographic studies on African savannah mammals have described three main clades: West/Central Africa, East Africa and Southern Africa, suggesting that there may have been major refugial areas in these regions during the more recent part of the Pleistocene climatic cycles (Hewitt 2004; Lorenzen *et al.* 2012). These three clades are clearly distinguishable in the lion based on mtDNA (nodes c, d and g in Figure 3) and autosomal data (Bertola *et al.*, submitted). A model-based study on the habitat suitability for mammals and birds during the last glacial maximum (LGM) suggests that there were five possible refugia sub-Saharan Africa: one in Upper Guinea, one or two in the

Cameroon Highlands – Congo Basin, one in the Ethiopian Highlands, one in Angola-Namibia, and one in East/Southern Africa (Levinsky *et al.* 2013). These areas represent the five sub-Saharan lion groups, described in this study, being West, Central, North East, South West, and East/Southern, respectively. In addition, we find corroboration in phylogeographic patterns from other savannah mammals with a distribution similar to that of the lion. Apart from the most basal dichotomy, shown for other species in Table 1 and Figure 1, the South West clade, which harbors lion populations from Angola and Namibia, is also recognized in giraffe (*Giraffa camelopardalis*) (Brown *et al.* 2007), zebra (*Equus zebra*) (Moodley & Harley 2006), impala (*Aepyceros melampus*) (Nersting & Arctander 2001), greater kudu (*Tragelaphus strepsiceros*) (Nersting & Arctander 2001) and sable antelope (*Hippotragus niger*) (Pitra *et al.* 2002). Within East Africa, the North East clade is also found in kob (*Kobus kob*) (Lorenzen *et al.* 2006), oryx (*Oryx beisa*) (Masembe *et al.* 2006), impala (*Aepyceros melampus*) (Nersting & Arctander 2001) and greater kudu (*Tragelaphus strepsiceros*) (Nersting & Arctander 2001). Finally, the distinction we find between the West and the Central African lion is also recognized in the phylogeographic pattern of roan antelope (*Hippotragus equinus*) (Alpers *et al.* 2004), potentially resulting from the lower Niger River as a permanent barrier for gene flow. Climatological events have also heavily influenced migration of early humans (Castañeda *et al.* 2009; Blome *et al.* 2012) and as a result, similar major clades and phylogeographic patterns are found in human datasets (Templeton 2002; Gonder *et al.* 2007; Tishkoff *et al.* 2009).

Phylogenetic variation within the six geographic groups of the modern lion appears to have mainly emerged within the last c. 100,000 year (100 kyr), including the cool last glacial (Marine Oxygen Isotope Stage (MIS) 4, 3 and 2) and two warmer periods (MIS 5 and 1) (Carto *et al.* 2009; Cronin 2010). Phylogenetic structure which had evolved in regional lineages during the previous glacial-interglacial cycles, mostly disappeared by c. 100 ka through various events, including genetic bottlenecks involving expansions and contractions from/to regional refugia (Migliore *et al.* 2013; Levinsky *et al.* 2013; Dauby *et al.* 2014). Since the HPD intervals are relatively large, we add a palaeoclimatic context to be able to propose a possible scenario that has contributed to the current phylogeographic pattern. The two major vegetation zones that likely influenced lion distribution through exclusion on the African continent are dry desert and dense rain forests (Nowell & Jackson 1996; Yamaguchi *et al.* 2004), both reflecting hydrological extremes. In the tropics the hydrological cycle is mainly driven by 21 kyr precession cycle of orbital climate forcing which is somewhat independent of the interglacial-glacial variations (Clement *et al.* 2004; Cronin 2010). The last coalescence between the North and South lineage (node a in Figure 3) in the lion is estimated at ~292 ka, positioned after the first cold interval (MIS 8.6 at 299 ka) of glacial MIS 8 (303-245 ka) (ages after (Imbrie *et al.* 1984)). This period is characterized by a maximum monsoon index, allowing dense wet forest to expand maximally northwards along an east-west axis in lower latitude Africa (Dupont & Hooghiemstra 1989; De Vivo & Carmignotto 2004; Kingdon 2007; Staver *et al.* 2011; Lehmann *et al.* 2011; Hardy *et al.* 2013; Dauby *et al.* 2014). Such vegetation pattern likely reduced or possibly eliminated the connection between northern and southern populations. The second oldest split between South West group and East/Southern & North East groups (node b in Figure 3) occurred at around ~231 ka, a moment positioned close to the first cool interval (MIS 7.4 at 228 ka) of interglacial MIS 7 (245-186 ka). The monsoon index was still high (Dupont & Hooghiemstra 1989) and the belt with rain forest may also in this interval have prevented a connection between lion populations, while simultaneously individuals belonging to the East/Southern group are distributed in a large area across East and Southern Africa (De Vivo & Carmignotto 2004; Barnett *et al.* 2006b). More recent radiation of the South West group (node g in Figure 3), estimated to have occurred ~114 ka, coinciding with the last part of the Eemian interglacial (MIS 5.5.), following a period of droughts, notably in the Kalahari region, in which suitable habitat was reduced in Southern Africa (Dupont 2011). The splits between East/Southern and North East Africa (node c in Figure 3), West Africa and Central & North Africa/Asia (node d in Figure 3), and Central and North Africa/Asia (node e in Figure 3) all appear to have occurred during MIS6 (186-128 ka) when relatively dry and cool conditions prevailed (Dupont & Hooghiemstra 1989; Petit *et al.* 1999). The splits in the North group are likely due to the periodically maximum north-south extension of the Sahara desert (Dupont & Hooghiemstra 1989; Hooghiemstra *et al.* 1992; Andel & Tzedakis 1996; Hoelzmann *et al.* 2004; De Vivo & Carmignotto 2004; Barnett *et al.* 2006b; Migliore *et al.* 2013). A connection between the North Africa/Asia group and the Central Africa group may have persisted during short periods that the monsoon front reached high latitudes, explaining the close genetic relationship to the North Africa/Asia clade (Dupont & Hooghiemstra 1989; Hooghiemstra *et al.* 1992; Hoelzmann *et al.* 2004). The West African population possibly became isolated and reduced in numbers by the significant southwards expansion of the Sahara during MIS 4 (71-59 ka) (Dupont & Hooghiemstra 1989; Hooghiemstra *et al.* 1992; Hoelzmann *et al.* 2004; Castañeda *et al.* 2009; Dupont 2011), and started radiating around 63 ka. There are no indications from our data that the current lion population in India was sourced or reinforced by introductions from sub-Saharan African lions, as was recently hypothesized (Thapar *et al.* 2013).

The deep ancestral split within the African lion and the topology of the phylogenetic tree, along with the nested position for the Asiatic subspecies, clearly illustrate and support the notion that the current taxonomic division does not reflect the evolutionary history of the lion. Consequentially, it hampers proper priority setting for lion conservation, particularly in West and Central Africa. Since the distinct genetic lineages within the African lion are further supported by nuclear data (Antunes *et al.* 2008; Bertola *et al.* submitted) and morphological data (Hemmer 1974; Mazák 2010), we suggest to recognize a northern subspecies, including West Africa, Central Africa and North Africa/Asia, and a southern subspecies, including the lineages North East, East/Southern and South West, in line with the proposed revision by Barnett *et al.* (2014). Within these two subspecies, the distinct phylogeographic clades should be managed as Evolutionary Significant Units (ESUs), sensu Moritz (1994) (Moritz 1994), in the absence of conflicting conclusions based on other genetic markers. Data from more nuclear loci, and from sampling locations at the geographical borders of the proposed haplogroup ranges may provide a better insight, but are not likely to change the main pattern as is described in this paper.

Our study shows a fine-scale phylogeographic pattern for the lion, with strongly significant support for a basal north-south dichotomy, as is also observed in other African savannah mammals. By analysing samples from more localities, the phylogenetic position of the Asiatic subspecies was resolved and it was possible to propose ranges and connectivity zones for six major phylogenetic clades: West Africa, Central Africa and North Africa/Asia (North group) and North East, East/Southern and South West (South group). In context with the timing of the nodes in the phylogenetic tree, our results contribute to understanding the evolutionary forces that shaped the genetic makeup of several African savannah mammals and the extant lion clades in particular. Current nomenclature of the lion, recognizing an African and an Asiatic subspecies, is not in line with the evolutionary history of the species; we therefore propose a revision of the current taxonomy distinguishing a northern and a southern subspecies.

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Data accessibility

All sequence data generated in this study have been submitted to GenBank. Accession numbers are listed in Supplemental Table S3.

Supporting information

Supporting information which is not included here may be found in the online version of this article and is available upon request.

Supplemental Table S1. Lion samples and outgroup sequences used in this study.

Number	Country	Location	Region	Source	Literature
1	Senegal	Niokolo-Koba NP	West	Wild	Bertola et al., 2014
2	Senegal	Niokolo-Koba NP	West	Wild	Bertola et al., 2014
3	Senegal	Niokolo-Koba NP	West	Wild	Bertola et al., 2014
4	Senegal	Niokolo-Koba NP	West	Wild	Bertola et al., 2014
5	Senegal	Niokolo-Koba NP	West	Wild	Bertola et al., 2014
6	Senegal	Niokolo-Koba NP	West	Wild	Bertola et al., 2014
7	Senegal	Niokolo-Koba NP	West	Wild	Bertola et al., 2014
8	Guinea	-	West	Wild	this study
9	Benin	Pendjari NP	West	Wild	this study
10	Benin	Pendjari NP	West	Wild	Bertola et al., 2011
11	Benin	Pendjari NP	West	Wild	Bertola et al., 2014
12	Benin	Pendjari NP	West	Wild	Bertola et al., 2014
13	Benin	Pendjari NP	West	Wild	Bertola et al., 2014
14	Nigeria	Yankari GR	West	Wild	this study
15	Nigeria	Yankari GR	West	Wild	this study
16	Nigeria	Yankari GR	West	Wild	this study
17	Nigeria	Yankari GR	West	Wild	this study
18	Nigeria	Kainji NP	West	Wild	this study
19	Nigeria	Kainji NP	West	Wild	this study
20	Cameroon	Waza NP	Central	Wild	Bertola et al., 2011
21	Cameroon	Waza NP	Central	Wild	this study
22	Cameroon	Waza NP	Central	Wild	Bertola et al., 2011
23	Cameroon	Waza NP	Central	Wild	Bertola et al., 2011
24	Cameroon	Waza NP	Central	Wild	Bertola et al., 2011
25	Cameroon	Waza NP	Central	Wild	Bertola et al., 2011
26	Cameroon	Waza NP	Central	Wild	Bertola et al., 2011
27	Cameroon	Waza NP	Central	Wild	Bertola et al., 2011
28	Cameroon	Waza NP	Central	Wild	Bertola et al., 2011
29	Cameroon	Faro NP	Central	Wild	this study
30	Cameroon	Faro NP	Central	Wild	this study
31	Cameroon	Bénoué NP	Central	Wild	this study
32	Cameroon	Bénoué NP	Central	Wild	Bertola et al., 2011
33	Cameroon	Bouba Njida NP	Central	Wild	this study
34	Cameroon	Bouba Njida NP	Central	Wild	this study
35	Cameroon	Bouba Njida NP	Central	Wild	this study
36	Chad	Zakouma NP	Central	Wild	Bertola et al., 2011
37	Chad	Zakouma NP	Central	Wild	Bertola et al., 2011
38	Chad	Zakouma NP	Central	Wild	Bertola et al., 2011
39	Chad	Zakouma NP	Central	Wild	Bertola et al., 2011
40	CAR	Birao	Central	Museum (I)/Wild	this study
41	DRC	Garamba NP	Central	Wild	Bertola et al., 2014
42	DRC	Garamba NP	Central	Wild	this study
43	DRC	Garamba NP	Central	Wild	Bertola et al., 2014
44	DRC	Garamba NP	Central	Wild	Bertola et al., 2014
45	DRC	Garamba NP	Central	Wild	Bertola et al., 2014
46	DRC	Garamba NP	Central	Wild	Bertola et al., 2014
47	DRC	-	Central	Captive (i)	this study
48	DRC	-	Central	Captive (i)	Bertola et al., 2011
49	DRC	Ruindi Plains S. of Lake Edward	Central	Museum (II)/Wild	this study
50	Ethiopia	Gambela NP	East	Wild	this study
51	Ethiopia	Kaffa Province	East	Wild	this study
52	Ethiopia	Nechisar NP	East	Wild	this study
53	Ethiopia	Nechisar NP	East	Wild	this study
54	Ethiopia	Bale Mountains NP	East	Wild	this study
55	Ethiopia	Oromia region, Hudet	East	Wild	this study
56	Ethiopia	Somali region, Dolo Ado	East	Wild	this study
57	Ethiopia	Somali region, Kebri Dehar	East	Wild	this study
58	Ethiopia	Somali region	East	Wild/Captive (ii)	this study
59	Ethiopia	Somali region	East	Wild/Captive (ii)	this study
60	Ethiopia	-	East	Captive (iii)	this study
61	Ethiopia	-	East	Captive (iii)	Bertola et al., 2014
62	Ethiopia	-	East	Captive (iii)	Bertola et al., 2014

Number	Country	Location	Region	Source	Literature
63	Ethiopia	-	East	Captive (iii)	Bertola et al., 2014
64	Ethiopia	-	East	Captive (iii)	Bertola et al., 2014
65	Ethiopia	-	East	Captive (iv)	Bertola et al., 2011
66	Ethiopia	-	East	Captive (iv)	Bertola et al., 2011
67	Ethiopia	-	East	Captive (iv)	Bertola et al., 2011
68	Ethiopia	-	East	Captive (iv)	Bertola et al., 2011
69	Somalia	-	East	Captive (v)	Bertola et al., 2011
70	Somalia	-	East	Captive (v)	Bertola et al., 2011
71	Somalia	-	East	Captive (vi)	this study
72	Kenya	Aberdare NP	East	Wild	this study
73	Kenya	Maasai Mara NR	East	Wild	this study
74	Kenya	Maasai Mara NR	East	Wild	this study
75	Kenya	Maasai Mara NR	East	Wild	this study
76	Kenya	Maasai Mara NR	East	Wild	this study
77	Kenya	Maasai Mara NR	East	Wild	this study
78	Kenya	Maasai Mara NR	East	Wild	this study
79	Kenya	Amboseli NP	East	Wild	Bertola et al., 2014
80	Kenya	Amboseli NP	East	Wild	Bertola et al., 2014
81	Kenya	Amboseli NP	East	Wild	Bertola et al., 2014
82	Kenya	Amboseli NP	East	Wild	Bertola et al., 2014
83	Kenya	Amboseli NP	East	Wild	Bertola et al., 2014
84	Kenya	Amboseli NP	East	Wild	Bertola et al., 2014
85	Kenya	Amboseli NP	East	Wild	Bertola et al., 2014
86	Kenya	Kuku group ranch	East	Wild	this study
87	Kenya	Tsavo East NP	East	Wild	this study
88	Kenya	Tsavo East NP	East	Wild	this study
89	Kenya	Tsavo East NP	East	Wild	this study
90	Kenya	Tsavo East NP	East	Wild	this study
91	Tanzania	Serengeti NP	East	Wild	Bertola et al., 2014
92	Tanzania	Serengeti NP	East	Wild	Bertola et al., 2014
93	Tanzania	Serengeti NP	East	Wild	Bertola et al., 2014
94	Tanzania	Ngorongoro Conservation Area	East	Wild	Bertola et al., 2014
95	Zambia	Mpika town	South	Wild	this study
96	Zambia	Mulobezi town	South	Wild	this study
97	Zambia	Mumbwa town	South	Wild	this study
98	Zambia	north of Lusaka	South	Wild	this study
99	Zambia	Luangwa valley	South	Wild	Bertola et al., 2014
100	Zambia	Luangwa valley	South	Wild	Bertola et al., 2014
101	Zambia	Luangwa valley	South	Wild	Bertola et al., 2014
102	Zambia	Luangwa valley	South	Wild	Bertola et al., 2014
103	Zambia	Luangwa valley	South	Wild	Bertola et al., 2014
104	Zambia	Luangwa valley	South	Wild	Bertola et al., 2014
105	Zambia	Luangwa valley	South	Wild	Bertola et al., 2014
106	Zambia	Luangwa valley	South	Wild	Bertola et al., 2014
107	Zambia	Luangwa valley	South	Wild	Bertola et al., 2014
108	Zambia	Luangwa valley	South	Wild	this study
109	Zambia	Luangwa valley	South	Wild	this study
110	Zambia	Luangwa valley	South	Wild	this study
111	Zambia	Victoria Falls	South	Museum (III)/Wild	this study
112	Zambia	Mweru Wantipa	South	Museum (IV)/Wild	this study
113	Zimbabwe	Save Valley Conservancy	South	Wild	this study
114	Zimbabwe	Save Valley Conservancy	South	Wild	this study
115	Zimbabwe	Save Valley Conservancy	South	Wild	this study
116	Zimbabwe	Save Valley Conservancy	South	Wild	this study
117	Zimbabwe	Nuanetsi area	South	Museum (V)/Wild	this study
118	Zimbabwe	Robin's Camp	South	Museum (V)/Wild	this study
119	Botswana	-	South	Captive (vii)	Bertola et al., 2011
120	Botswana	-	South	Captive (vii)	this study
121	Botswana	-	South	Captive (vii)	Bertola et al., 2011
122	Botswana	Aha Hills	South	Museum (III)/Wild	this study
123	Mozambique	Caia	South	Museum (VI)/Wild	this study
124	Mozambique	Changara	South	Museum (III)/Wild	this study
125	Angola	-	South	Museum (VI)/Wild	this study
126	Namibia	Etosha NP	South	Wild	Bertola et al., 2014
127	Namibia	Etosha NP	South	Wild	Bertola et al., 2014
128	Namibia	Easter Caprivi	South	Wild	this study
129	Namibia	East Etosha	South	Wild	this study
130	Namibia	East and West Caprivi/Botswana	South	Wild	this study
131	Namibia	Eastern Etosha	South	Wild	this study
132	Namibia	Eastern Etosha	South	Wild	this study
133	Namibia	West Caprivi and Angola	South	Wild	this study
134	Namibia	Etosha Central	South	Wild	this study
135	Namibia	East Etosha	South	Wild	this study
136	Namibia	Erongo/Walvis Bay	South	Museum (II)/Wild	this study
137	Namibia	-	South	Captive (viii)	this study
138	Namibia	-	South	Captive (viii)	Bertola et al., 2011
139	RSA	Kalahari Gemsbok NP	South	Wild	Bertola et al., 2014
140	RSA	Kalahari Gemsbok NP	South	Wild	Bertola et al., 2014
141	RSA	Kgalagadi to Tswalu	South	Wild	this study
142	RSA	Kgalagadi to Tswalu	South	Wild	this study

Number	Country	Location	Region	Source	Literature
143	RSA	Kgalagadi Transfrontier Park	South	Wild	this study
144	RSA	Kgalagadi Transfrontier Park	South	Wild	this study
145	RSA	Kruger NP: Gogonthaba, Malelane	South	Wild	Bertola et al., 2014
146	RSA	Kruger NP: Gogonthaba, Malelane	South	Wild	Bertola et al., 2014
147	RSA	Kruger NP: Skukuza Phabeni/ Nwaswitshaka watergat pad junction	South	Wild	Bertola et al., 2014
148	RSA	Kruger NP: Skukuza Phabeni/ Nwaswitshaka watergat pad junction	South	Wild	Bertola et al., 2014
149	RSA	Kruger NP: Pretoriuskop, Fayi loop	South	Wild	Bertola et al., 2014
150	RSA	Kruger NP: Lower Sabie, S128	South	Wild	Bertola et al., 2014
151	RSA	Kruger NP: Crocodile Bridge	South	Wild	Bertola et al., 2014
152	RSA	Kruger NP: Crocodile Bridge	South	Wild	Bertola et al., 2014
153	RSA	Kruger NP: Stolznek, North of Biyamiti	South	Wild	Bertola et al., 2014
154	RSA	Kruger NP: Stolznek	South	Wild	Bertola et al., 2014
155	RSA	Venetia-Limpopo NR, Tuli Block	South	Wild	this study
156	RSA	Venetia-Limpopo NR, Tuli Block	South	Wild	this study
157	RSA	Venetia-Limpopo NR, Tuli Block	South	Wild	this study
158	RSA	Venetia-Limpopo NR, Tuli Block	South	Wild	this study
159	RSA	Venetia-Limpopo NR, Tuli Block	South	Wild	this study
160	RSA	Venetia-Limpopo NR, Tuli Block	South	Wild	this study
161	RSA	Kruger NP: Timbavati	South	Captive (ix)	Bertola et al., 2011
162	RSA	Kruger NP: Timbavati	South	Captive (ix)	this study
163	RSA	Kruger NP: Timbavati	South	Captive (ix)	Bertola et al., 2011
164*	RSA	-	South	Museum (I)/Wild	this study
165	Barbary	-	North	Museum (II)/Wild	this study
166	Barbary	-	North	Museum (VI)/Wild	this study
167*	Middle-East	-	North	Museum (VII)/Captive	this study
168*	Middle-East	-	North	Museum (VII)/Captive	this study
169	India	Gir Forest NP	India	Wild	Bertola et al., 2014
170	India	Gir Forest NP	India	Wild	Bertola et al., 2014
171	India	Gir Forest NP	India	Wild	Bertola et al., 2014
172	India	Gir Forest NP	India	Wild (Captive born (x), founders both Wild)	Bertola et al., 2014
173	India	Gir Forest NP	India	Wild (Captive born (x), founders both Wild)	Bertola et al., 2014
174	India	Gir Forest NP	India	Wild (Captive born (x), founders both Wild)	this study
175	India	Gir Forest NP	India	Captive (i)	Bertola et al., 2011
176	India	Gir Forest NP	India	Captive (i)	Bertola et al., 2011
177	India	Gir Forest NP	India	Captive (i)	Bertola et al., 2011
178	India	Gir Forest NP	India	Captive (xi)	Bertola et al., 2011
179	Senegal (PL1)	-	West	GenBank	Barnett et al., 2006+2014
180	Senegal (PL2)	-	West	GenBank	Barnett et al., 2006+2014
181	Barbary (PL3)	-	North	GenBank	Barnett et al., 2006+2014
182	Burkina Faso (PL4)	-	West	GenBank	Barnett et al., 2006+2014
183	Tunisia (PL5)	-	North	GenBank	Barnett et al., 2006+2014
184	North Africa (PL6)	-	North	GenBank	Barnett et al., 2006+2014
185	Algeria (PL7)	-	North	GenBank	Barnett et al., 2006+2014
186	Iran (PL8)	-	North	GenBank	Barnett et al., 2006+2014
187	Iran (PL9)	-	North	GenBank	Barnett et al., 2006+2014
188	North Africa "Tower of London" (PL11)	-	North	GenBank	Barnett et al., 2006+2014
189	North Africa "Tower of London" (PL12)	-	North	GenBank	Barnett et al., 2006+2014
190	Sudan (PL13)	-	Central	GenBank	Barnett et al., 2006+2014
191	CAR (PL15)	-	Central	GenBank	Barnett et al., 2006+2014
192	CAR (PL16)	-	Central	GenBank	Barnett et al., 2006+2014
193	Asiatic lion (*Panthera leo persica*)	India	Support 16 mitochondrial regions	GenBank	Bagatharia et al., 2013
194	Asiatic lion (Panthera leo persica)	India	Support 16 mitochondrial regions	GenBank	Bagatharia et al., 2013
195	Cave lion (*Panthera leo spelaea*)	Germany	Outgroup	GenBank	Burger et al. (2004) + Barnett et al. (2009)
196	Leopard (*Panthera pardus*)	Amur	Outgroup	Captive (xi)	this study
197	Leopard (*Panthera pardus*)	Unknown	Outgroup	GenBank	Wei et al. (2011)
198	Tiger (*Panthera tigris*)	Bengal	Outgroup	GenBank	Kitpipit et al. (2011)
199	Tiger (*Panthera tigris*)	Bengal	Outgroup	GenBank	Kitpipit et al. (2011)
200	Tiger (*Panthera tigris*)	Sumatra	Outgroup	GenBank	Kitpipit et al. (2011)
201	Tiger (*Panthera tigris*)	Sumatra	Outgroup	GenBank	Kitpipit et al. (2011)
202	Tiger (*Panthera tigris*)	Amur	Outgroup	GenBank	Kitpipit et al. (2011)
203	Tiger (*Panthera tigris*)	Amur	Outgroup	GenBank	Kitpipit et al. (2011)
204	Snow leopard (*Panthera uncia*)	Unknown	Outgroup	GenBank	Wei et al. (2011)
205	Clouded leopard (*Neofelis nebulosa*)	Unknown	Outgroup	GenBank	Wu et al. (2007)

* Excluded from analyses presented in the main text. See Supplemental Information 2 for background information and additional analyses.

SOURCE	**MUSEUM**	I: Zoological Museum, University of Amsterdam, Amsterdam, The Netherlands / Naturalis Biodiversity Center, Leiden, The Netherlands
		II: Swedish Museum of Natural History, Stockholm, Sweden
		III: Smithsonian: Smithsonian Institution, Washington D.C., U.S.A.
		IV: Brussels: Royal Belgian Institute of Natural Sciences, Brussels, Belgium
		V: Bulawayo: Natural History Museum of Zimbabwe, Bulawayo, Zimbabwe
		VI: Naturalis Biodiversity Center, Leiden, The Netherlands
		VII: Humboldt: Museum für Naturkunde (MfN)/Humboldt Museum, Berlin, Germany
	CAPTIVE	i: Diergaarde Blijdorp, Rotterdam, The Netherlands
		ii: BornFree Ethiopia, lions confiscated from the Presidential Palace in Addis Ababa, Ethiopia
		iii: Addis Ababa Lion Zoo, Addis Ababa, Ethiopia
		iv: Sanaa Zoo, Sanaa, Yemen
		v: Safaripark Beekse Bergen, Hilvarenbeek, The Netherlands
		vi: Confiscated individual, Breeding Centre, UAE
		vii: Dierenpark Amersfoort, Amersfoort, The Netherlands
		viii: Zoo Basel, Basel, Switserland
		ix: Ouwehands dierenpark, Rhenen, The Netherlands
		x: Sakkarbaug Zoo: Sakkarbaug Zoological Garden, Junagadh, Gujarat, India
		xi: Planckendael: Planckendael, Muizen, Belgium

Supplemental Table S2. (online only) Overview of processing of lion samples included and accompanying Genbank accession numbers.

Supplemental Table S3. Primers used in this study.

Primer Set1	**3 Primers**		
Primer name	**Annealing Temp**	**Sequence (5'-3')**	**Reference**
1F	50-55 ˚C	CGTTGTACTTCAACTATAAGAACTT	Bertola et al., 2011
1R	50-55 ˚C	ATGGGATTGCTGATAGGAGATTAG	Bertola et al., 2011
2F	53-55 ˚C	GTGGGGCCAAATATCCTTTT	Bertola et al., 2011
4R	53-55 ˚C	TTTTTGGTTTACAAGACCAAGGTA	Bertola et al., 2011
5F	53-55 ˚C	AAATCGCCTCCTCAAATGAA	Bertola et al., 2011
5R	53-55 ˚C	AATATTCATGGGAGGGCAGTC	Bertola et al., 2014
Primer Set2	**5 Primers**		
Primer name	**Annealing Temp**	**Sequence (5'-3')**	**Reference**
1F	50-55 ˚C	CGTTGTACTTCAACTATAAGAACTT	Bertola et al., 2011
1R	50-55 ˚C	ATGGGATTGCTGATAGGAGATTAG	Bertola et al., 2011
2F	51-53 ˚C	GTGGGGCCAAATATCCTTTT	Bertola et al., 2011
2R	51-53 ˚C	GAAGGCCTAGGATATCTTTGATTG	Bertola et al., 2014
3F	51-53 ˚C	GACTCAGATAAAATTCCATTCCA	Bertola et al., 2014
3R	51-53 ˚C	CATTATTCCTCGCTGTTTGG	Bertola et al., 2014
4F	51-53 ˚C	CAATTATCCCTGCCCTCCA	Bertola et al., 2014
4R	51-53 ˚C	TTTTTGGTTTACAAGACCAAGGTA	Bertola et al., 2011
5F	53-55 ˚C	AAATCGCCTCCTCAAATGAA	Bertola et al., 2011
5R	53-55 ˚C	AATATTCATGGGAGGGCAGTC	Bertola et al., 2014
Primer Set3	**12 Primers**		
Primer name	**Annealing Temp**	**Sequence (5'-3')**	**Reference**
aDNA1F	50 ˚C	CGTTGTACTTCAACTATAAGAACTT	this study
aDNA1R	50 ˚C	CTAGAAAGAGGCCGGTGAGAA	this study
aDNA2F	50 ˚C	GCTCCTTATTAGGAGTATGCTTAATCC	this study
aDNA2R	50 ˚C	CATGCATGTATAGGCAGATAAAGA	this study
aDNA3F	50 ˚C	TGGCTGAATTATCCGGTACCTA	this study
aDNA3R	50 ˚C	GCACCTCAAAAGGATATTTGG	this study
aDNA4F	50 ˚C	AGCTACAGCCTTCATAGGATATGT	this study
aDNA4R	50 ˚C	TGGAAGGATGAAGTGGAAGG	this study
aDNA5F	50 ˚C	GGAGGCTTCTCAGTAGACAAAG	this study
aDNA5R	50 ˚C	TGATTGTATAGTATGGATGGAATGG	this study
aDNA6F	50 ˚C	CCCCTCAGGAATGGTATCTG	this study
aDNA6R	50 ˚C	ATATGGGGAGGGGTGCTTAG	this study
aDNA7F	50 ˚C	CTCACCAGACCTATTAGGAGATCC	this study
aDNA7R	50 ˚C	GAGGGCAGGGATAATTGCTA	this study
aDNA8F	50 ˚C	GCAATCCTCCGATCTATTCC	this study
aDNA8R	50 ˚C	CCAATTCATGTCAGGGTCAG	this study
aDNA9F	50 ˚C	CTTATTCTGATTCCTAGTAGCGGA	this study
aDNA9R	50 ˚C	CGTTCTCCTTTTTTGGTTTACAAG	this study
aDNA10F	50 ˚C	GCCTCCTCAAATGAAGAGTCT	this study
aDNA10R	50 ˚C	TGCAATATATGAATTGTGAAAGTTACG	this study
aDNA11F	50 ˚C	GCACCCAAAGCTGAAATTCT	this study
aDNA11R	50 ˚C	TCACTTGCTTTTCGTGGGG	this study
aDNA12F	50 ˚C	CTGTGCTTGCCCAGTATGTC	this study
aDNA12R	50 ˚C	CTGTACATGCTTAATATTCATGGG	this study
Primer Set LR	**4 Primers**		
Primer name	**Annealing Temp**	**Sequence (5'-3')**	**Reference**
LR 1F (NADH4)	56 ˚C	CTCACTTTCTGCACCTCTACTAGTCTTA	this study
LR 1R (16S)	56 ˚C	ACGGATCAGAAGTAAGAGACAGTAAAG	this study
LR 2F (16S)	56 ˚C	CATCACCTCTAGCATTTCCAGTATTAG	this study
LR 2R (NADH4)	56 ˚C	ACTAGCCATGAGCATTAGTGGTAGG	this study

Supplemental Table S4. Results of estimates for divergence times for lion clades in years ago (ya), compared to estimates from previous publications. Constraints include the approach and calibration points used. Names of the clades refer to the ingroups.

		This study	**Burger et al., 2004**	**Antunes et al., 2008**	**Barnett et al., 2014**
Software		BEAST	r8s	Lintree	BEAST
Data		complete mtDNA (total: 17 kb) + 1.4 kb fragments	mtDNA (total: 1.1 kbp)	mtDNA (total: 1.9 kbp)	mtDNA (total: 1.1 kbp)
Constraints		0.55 million ya (Cave lion - Lion); 1.6 million ya (Leopard-Lion); 3.8 million ya (Panthera)	clock-based estimate	clock-based estimate	0.55 million ya (Cave lion - Lion)
	Node (Fig. 3)	**Age of nodes (95% HPD)**			
Panthera	not shown	3.443 million ya (2.590-4.373)	1.428–2.295 million ya		
Leopard - Lion	not shown	1.469 million ya (1.135-1.812)	1–1.559 million ya		
Cave lions - Lion	not shown	556,900 ya (510,800-606,100)			
Lion	a	291,700 ya (178,000-417,700)	74,000-203,000 ya	324,000 ya (145,000-502,000)	124,200 ya (81,800-183,500)
South	b	231,300 ya (132,300-338,700)		split not detected	81,900 ya (45,700-122,200)
East/Southern - North East	c	183,700 (107,000-271,100)		split not detected	57,800 ya (26,800-96,600)
North	d	174,700 ya (94,900-276,700)		118,000 ya (28,000-208,000)	61,500 ya (32,700-97,300)
Central - India	e	141,100 ya (71,700 - 216,600)		split not detected	split not detected
India - North Africa	f	110,800 ya (39,700-200,100)		split not detected	21,100 ya (8,300-38,800)
South West	g	113,800 ya (55,200-189,000)		169,000 ya (34,000-304,000)	
East/Southern	h	78,100 ya (37,200-132,100)		101,000 ya (11,000-191,000)	
North East	i	63,900 ya (18,000-118,600)		split not detected	
West - India	-	split not detected		split not detected	51,000 ya (26,600-83,100)
West	j	63,400 ya (15,100-129,100)		split not detected	
Central	k	49,600 ya (20,700-91,000)		split not detected	

Supplemental Information S1. Details on sample storage and processing.

Samples were preserved dried, in 95% ethanol or in buffer (0.15 M NaCl, 0.05 M Tris-HCl, 0.001 M EDTA, pH = 7.5) and stored at -20°C (Supplemental Table 4). For blood and tissue samples DNA was extracted using the DNeasy Blood & Tissue kit (Qiagen) following the manufacturer's protocol. For the scat and the museum samples, a protocol for aDNA extractions from bone and teeth (Rohland & Hofreiter 2007) was followed. In all cases a mock extraction was included to check for contamination. All museum samples were processed in the aDNA facility of DNAmarkerpoint, Leiden University, which is physically isolated from other laboratories and where no previous work on felids had been conducted. In addition, two scat samples which contained strongly degraded DNA, 8.Guinea and 30.Cameroon, were included in the aDNA procedure. Before each extraction, the surfaces in the extraction room were cleaned using 10% bleach and all materials were cleaned and irradiated with UV light for a minimum of one hour.

The complete mitochondrial genome was analyzed for ten individuals by sequencing on an Illumina HiSeq2000 using 99 bp paired-end sequencing with 200-400 bp insert size (Leiden Genome Technology Center, Leiden, The Netherlands). In the first run, two individuals (9.Benin and 89.Kenya) were tagged and pooled with leopard DNA (ratios 1:1:2 for 9.Benin, 89.Kenya and 179.Leopard respectively). In the following two runs, four individuals (21. Cameroon+71.Somalia+162.RSA+ 174.India and 42.DRC+95.Zambia+96.Zambia+131.Namibia) were tagged and equimolarily pooled. Resulting reads were identified based on the unique adapter sequences.

For four individuals the complete mtDNA was analysed by performing two long range PCRs for amplifying all ~18,000 bp. Primers were designed based on known leopard sequences available on Genbank using Primer3v 0.4.0 (Rozen & Skaletsky 2000). Primer sites were chosen such that the forward and corresponding reverse primer were not both located in one of the known numts that have been identified in felids (Lopez *et al.* 1996; Cracraft *et al.* 1998; Kim *et al.* 2006). For amplification either the LA PCR kit (TaKaRa) or the GoTaq Long PCR Master Mix (Promega) was used (Supplemental Table S2). Resulting PCR products were cut out from the gel, cleaned with the Wizard SV gel and PCR Clean-Up kit (Promega) and sonically fragmented. Barcoded Libraries for sequencing were prepared from the fragmented PCR products using the Rapid Library Preparation Kit (Roche). Emulsion PCR and sequencing were performed on the 454/Roche FLX Genome Sequencer Titanium (Forensic Laboratory for DNA Research, Leiden, The Netherlands) according to the protocol.

Cytochrome b, tRNAThr, tRNAPro and the left domain of the control region (hereafter referred to as cytB+ctrl reg.) were amplified using three primer pairs in high quality blood and tissue samples, five primer pairs in the scat samples and twelve primer pairs in the aDNA samples. See Supplemental Table S3 for primer sequences. All primers were designed using the web-based software Primer3v 0.4.0 (Rozen & Skaletsky 2000). The modern samples were amplified using Taq DNA Polymerase (Invitrogen) or Phire Hot Start II DNA Polymerase (Thermo Scientific), depending on the amplification success. Annealing temperature was adjusted according to primer pair and according to previous PCR results (for details see Supplemental Table S2). The museum samples were amplified using AmpliTaq Gold DNA Polymerase (Invitrogen) and following a half-nested approach: in the first round (40 cycles) primer aDNA1F was combined with primer aDNA2R and a 1:50 dilution of the PCR product was used as a template for a second round PCR (40 cycles), in which primer aDNA1F was combined with aDNA1R and primer aDNA2F was combined with aDNA2R etc. In all cases multiple negative PCR controls were included to check for contamination.

Sequencing of the short, non-aDNA PCR products was performed by Macrogen Inc., Amsterdam, The Netherlands. The aDNA samples were sequenced on the Roche/454 platform (Forensic Laboratory for DNA Research, Leiden, The Netherlands). The 12 PCR products for each museum sample were equimolarily pooled, and after a test run containing one sample, the remaining 17 samples were divided in two pools, which were analysed in two separate runs. To check for contamination and to distinguish the samples after sequencing, a unique combination of tags attached to the primers was used for each individual. In addition to the 454 sequencing, 22 PCR products were cloned to confirm sequences with a coverage <10 or inconclusive results (i.e. called base supported by <90% of available reads). Cloning was performed using the Invitrogen TOPO cloning kit following the manufacturer's protocol. From each cloned PCR product, between three and eight colonies were picked. Picked colonies were lysed by heating the cells in 30 µl of water for 10 minutes (min) at 95 °C. Cell lysates were amplified with M13 primers using the following PCR: 2 µl $MgCl_2$ (25 mM), 2 µl 10× PCR buffer, 0.25 µl dNTPs (2.5 mM each), 0.24 µl Taq polymerase, 0.5 µl M13 primers (10 µM each), and 2 µl cell lysate, with water added to a final volume of 20 µl. The PCR program was: 94 °C for 5 min followed by 40 cycles of 94 °C for 30 seconds (s), 55 °C for 45 s, 72 °C for 45 s and a final extension step of 72 °C for 4 min. The PCR products were sequenced by Macrogen Inc., Amsterdam, The Netherlands. Overlap between independent PCR products were used to check for DNA damage and sequencing errors. Unique point mutations (i.e. observed in a single sample) were checked by an independent PCR and sequencing for modern samples, or cloning for aDNA samples.

Read data from Illumina and 454 platforms were analysed using CLC Genomics (CLCBio). A leopard mitochondrial genome available on GenBank (EF551002.1) was used as reference. Mapping was performed by using default settings, except for length fraction and similarity fraction, which were increased to 0.8 and 0.85 respectively. Consensus sequences were extracted and aligned visually with Macrogen sequences. Since we observed one region that seemed to be absent in all Illumina samples, but present in all sequences derived by PCR and Sanger sequencing, and another region where the opposite was true, we constructed a new reference sequence and repeated the mapping of all Illumina and 454 reads, which lead to a more consistent coverage across the reference sequence. Sequences covering cytB+ctrl reg. that had already been analysed in earlier publications (Barnett *et al.* 2006a; Barnett *et al.* 2006b; Barnett *et al.* 2014; Bertola *et al.* 2011; Bertola *et al.* submitted) were added to the dataset for phylogenetic analyses.

Since Roche/454 sequencing does not perform well with mononucleotide repeats, all mononucleotide repeats of >3 bp were manually checked. Gaps resulting from inconclusive base calling were substituted by an ambiguous nucleotide. This was also done for inconclusive results on six positions in three aDNA samples which could not be resolved and a 62bp region with insufficient coverage in sample 165.Barbary. Two repetitive regions in the control region, RS-2 and RS-3, were excluded from the analysis, since aligning was difficult and the region is known to be heteroplasmic (Jae-Heup *et al.* 2001). In addition, a mononucleotide repeat of cytosines of variable length was excluded due to unknown homology (bp 1382-1393 in cytB+ctrl reg.). For phylogenetic analysis 179.Leopard was used as an outgroup and supplemented by six sequences from Genbank: clouded leopard (*Neofelis nebulosa*: DQ257669.1), snow leopard (*Panthera uncia*: EF551004.1), two sequences of tiger (*Panthera tigris*: JF357968.1 (Bengal) and JF357974.1 (Amur)), one sequence of leopard (*Panthera pardus*: EF551002.1) and one sequence of cave lion (*Panthera leo spelaea*: KC701376.1 + DQ899901.1). In addition, two complete mitochondrial genomes from Asiatic lions were included (JQ904290.1 and KC834784.1) (not included in Figures). Since the sequences from Genbank did not align well in the control region, likely due to the assembly method, this region of the Genbank sequences was replaced by ambiquous nucleotides to eliminate the influence of assembly quality.

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Supplemental Information S2. Data authenticity.

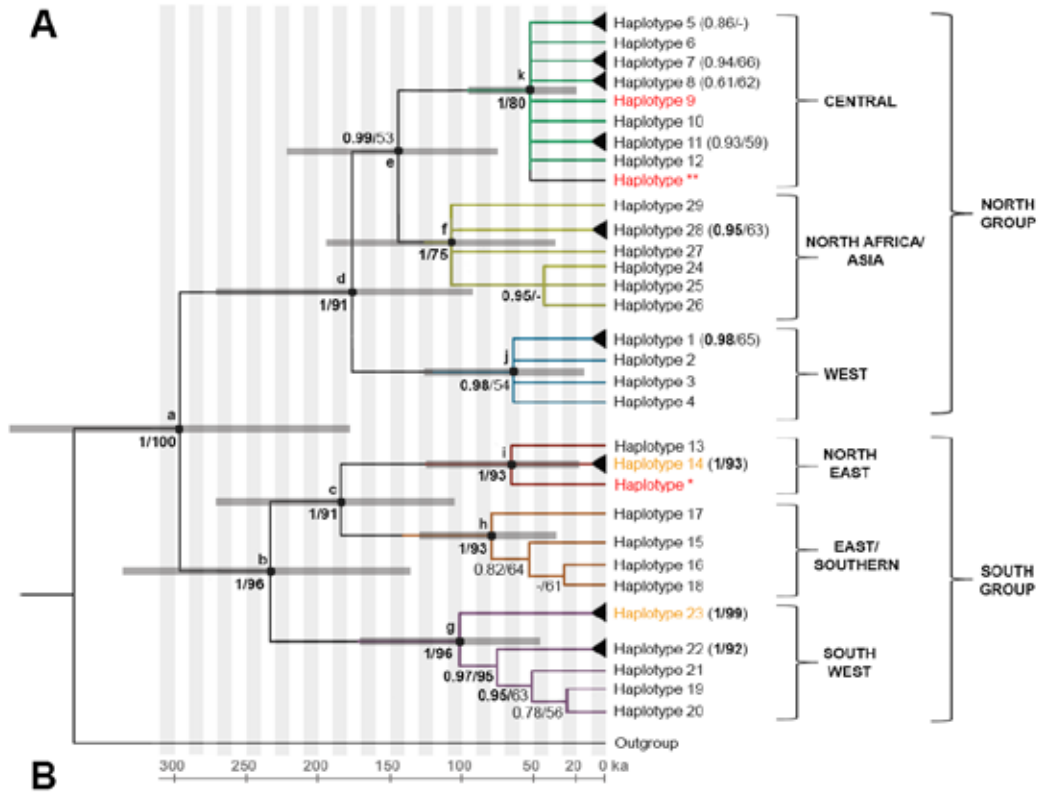
Samples from zoos and museums were only included in our study when sufficient information was available on the origin of the individual or its free-ranging ancestors. In four cases, samples showed unexpected results from the phylogenetic analyses. Since the origin of three samples could not be reconfirmed, they were excluded from analyses presented in the main text. For completeness, results of the analyses including these samples and captive popultions are shown below. In all cases, unique point mutations were double checked by independent PCR and sequencing and laboratory procedures were checked to exclude the possibility of contamination. Addition of these samples does not change the conclusions presented in the main text.

Haplotype 14: Ethiopia captive population (65-68 Ethiopia). This population is located on a long branch, clustering with the North East group. Despite relatively dense sampling of the region, no intermediate haplotypes were identified. Clustering based on mtDNA data and microsatellite data do not contradict the origin of these samples (Bertola *et al.*, submitted). These data were therefore included in all analyses.

Haplotype 23: Namibia captive population (137-138.Namibia). This population is located on a long branch in the South West group, with undetected intermediate haplotypes. Phylogenetic analyses place the population on the expected branch, in the South West group. These data were therefore included in all analyses.

Museum sample 164.RSA (Haplotype *). This sequence was placed in the North East group, with data from Ethiopia, Somalia and Central Kenya. Apart from this specimen, all included samples from the southern part of Kenya and further southward cluster with either East/Southern or the South West group. No samples from the North East group had been processed parallel to this sample and therefore we exclude the possibility of contamination. The specimen was collected by the late L. de Beaufort and comparing this entry to other specimen collected by L. de Beaufort, this entry contained very little information. Because of doubts regarding the authenticity of this entry, and the unexpected position in the phylogenetic tree, this sample was excluded from the phylogenetic analyses presented in the main text. Results for Bayesian, Maximum Likelihood, Network and BEAST analyses including this sample are shown below (Supplemental Figures S2-1 and S2-2).

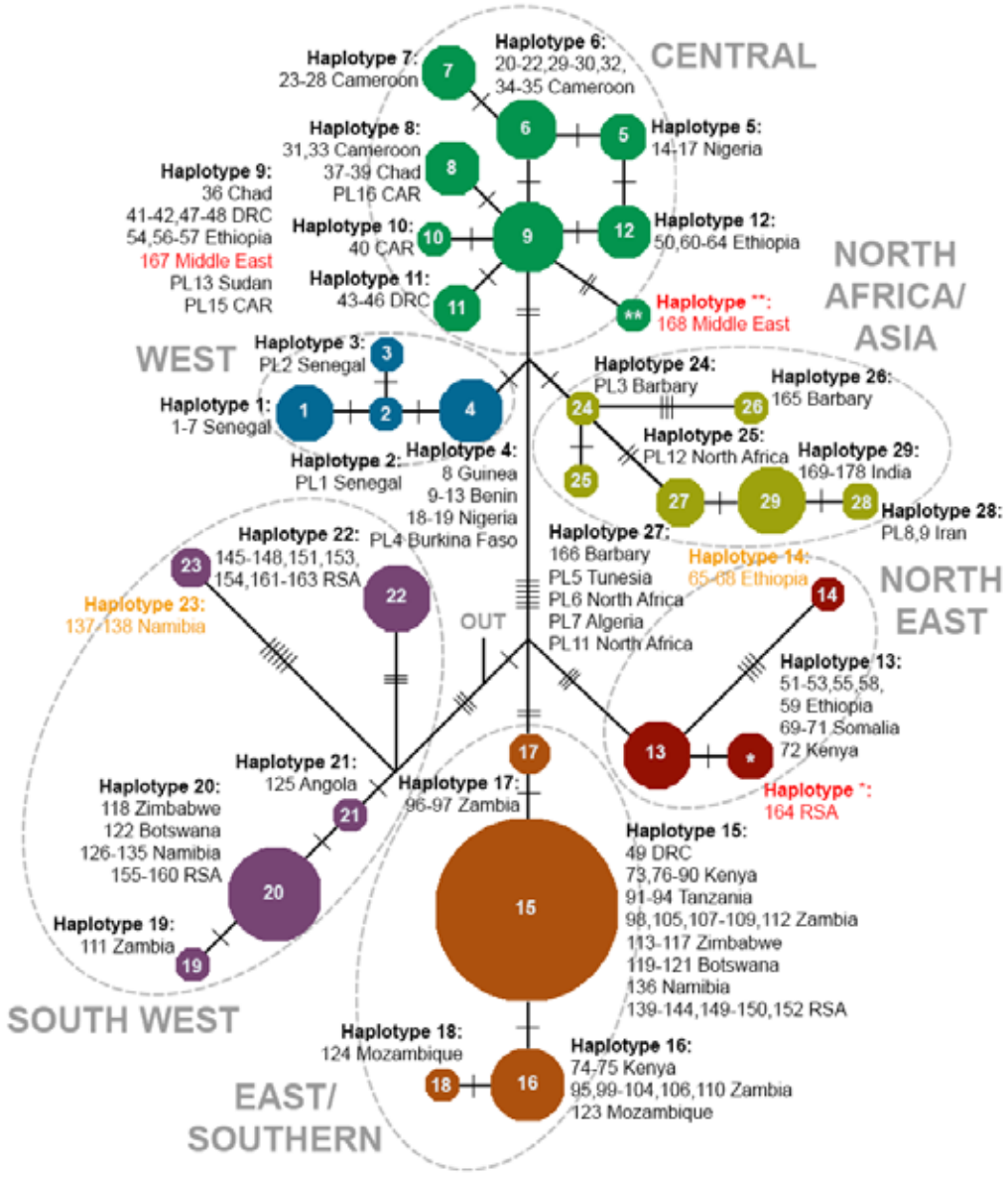
Museum samples 167-168.Middle East (Haplotype 9 and **): these specimen were labeled as hybrids between an Abyssinian male and a female from Mesopotamia (first generation zoo animals). They share a haplotype or cluster close to a haplotype from Central Africa. In contrast, the remaining ten sequences from North Africa and Iran cluster strongly with the Asiatic subspecies. No samples from the Central Africa group had been processed parallel to this sample and therefore we exclude the possibility of contamination. Regarding the sparse information about zoo populations in those times and the unexpected position in the phylogenetic tree, these specimen were excluded from the phylogenetic analyses presented in the main text. Haplotype 9 was retained, since this was found in several other samples from Central Africa. Results for Bayesian, Maximum Likelihood, Network and BEAST analyses including this sample are shown below (Supplemental Figures S2-1 and S2-2).



B

Clade	Node	Date (95% HPD)	Clade	Node	Date (95% HPD)	Clade	Node	Date (95% HPD)	Clade	Node	Date (95% HPD)
Modern Lion	a	293.8 ka (178.6-416.3)	North	d	176.8 ka (92.9-271.8)	South West Africa	g	1.4 ka (43.4-170.2)	West Africa	j	63.4 ka (15.0-127.6)
South	b	231.2 ka (138.7-337.2)	Central Africa - India	e	142.0 ka (75.1 - 222.8)	East/ Southern Africa	h	78.1 ka (34.8-129.7)	Central Africa	k	51.7 ka (19.7-95.0)
East/Southern - North East Africa	c	182.3 ka (104.3-271.4)	India - North Africa	f	106.4 ka (35.8-194.2)	North East Africa	i	65.1 ka (18.1-124.5)			

Supplemental Figure 2-1. Phylogenetic analyses for the complete lion dataset, including sixteen mitochondrial genomes and 178 cytb+ctrl reg. sequences A: Phylogenetic tree of lion populations throughout their complete geographic range, based on complete mitochondrial genomes and cytB+ctrl reg. sequences. Branch colours correspond to haplotype colours in Supplemental Figure 2-2. Populations mentioned above as long branches with missing intermediate haplotypes, are indicated in orange. Populations with limited information regarding their origin, which were excluded from analyses presented in the main text, are shown in red. Support is indicated as posterior probability (Bayesian analysis)/bootstrap support (ML analysis). Branches with a single haplotype have been collapsed to improve readability. Support for these branches is indicated by a black triangle at the tip of the branch (support shown in the label). Nodes which have been included for divergence time estimates are indicated with letters and 95% HPD node bars. Distance to outgroup and nodes without dated split is not in proportion to divergence time. B: divergence time estimates and 95% HPD from BEAST analysis, also indicated as error bars in Supplemental Figure 2-1A.



Supplemental Figure 2-2. Haplotype network based on cytB+ctrl reg. sequences of lions throughout their entire geographic range. Dashed lines indicate the groups discerned Bayesian/ML analysis in Supplemental Figure 2-1A. Populations indicated above as long branches with missing intermediate haplotypes, are shown in orange. Populations with limited information regarding their origin, which were excluded from analyses presented in the main text, are indicated in red. Haplotype size is proportional to its frequency in the dataset. Hatch marks represent a change in the DNA sequence. The connection to outgroup species is indicated by "OUT".

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