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

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### **Citation**

Bertola, L. D. (2015, March 18). *Genetic diversity in the lion (*panthera leo* (Linnaeus 1758)) : unravelling the past and prospects for the future*. Retrieved from <https://hdl.handle.net/1887/32419>

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

**Issue Date:** 2015-03-18



## Autosomal and mtDNA markers reveal concordant phylogenetic patterns of lion populations over the entire geographic range

(under review)

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### Abstract

The evolutionary history of a species is key for understanding the taxonomy and for the design of effective management strategies for species conservation. The knowledge about the evolutionary history of the lion (*Panthera leo*) is largely based on mitochondrial markers. Here, we investigate whether autosomal markers are concordant with previously described phylogeographic patterns. Special emphasis is placed on the lion in West/Central Africa, as previous studies using only mitochondrial markers have shown this region to hold a distinct evolutionary lineage. In addition, anthropogenic factors have led to a strong decline in West/Central African lion numbers in recent history, thus, the conservation value of these populations is particularly high. Analysis of 20 microsatellites and 1,454 bp of the mitochondrial DNA in 16 lion populations representing the entire geographic range of the species, found congruence in both types of markers, identifying four clusters: 1) West/Central Africa, 2) East Africa, 3) Southern Africa and 4) the Asiatic subspecies. This is not in line with the current taxonomy, which only recognizes an African and an Asiatic subspecies. There are no indications that genetic diversity in West/Central Africa lions is lower than in either East or Southern Africa, however, given this genetic distinction and the recent declines of lion numbers in this region, we strongly recommend prioritization of conservation projects in West/Central Africa. As the current taxonomic nomenclature does not reflect the evolutionary history of the lion, we suggest that a taxonomic revision of the lion is warranted.

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

## Introduction

Identifying and describing patterns of mitochondrial (mtDNA) and nuclear genetic variation is a crucial component to fully understanding the evolutionary history of a species. High quality phylogeographic data that represent the underlying genetic complexity are important for taxonomy and contribute to designing effective conservation strategies. This is of particular importance for species such as the lion (*Panthera leo*) that occupy large geographic ranges within which disjunct populations may not allow for natural dispersal and gene flow. Increasing habitat fragmentation and variable anthropogenic factors have created a growing need to manage lions at the population level (Riggio *et al.* 2012). In addition, several recent publications have sparked the discussion whether the current taxonomic nomenclature for the lion is justified (Bertola *et al.* 2011a; Dubach *et al.* 2013; Barnett *et al.* 2014).

Two subspecies of lion are currently recognized by the IUCN: the African lion (*Panthera leo leo*), ranging throughout sub-Saharan Africa with the exception of dense rain forest, and the Asiatic lion (*Panthera leo persica*), which exists as a single population in the Gir forest, India. Although all African lion populations are considered as belonging to the African subspecies, distinct genetic subgroups have been recognized (Dubach *et al.* 2005, 2013; Barnett *et al.* 2006a; b, 2014; Antunes *et al.* 2008; Bertola *et al.* 2011a; Bruche *et al.* 2012). Based on phylogenetic analysis of mitochondrial haplotypes only, lions in West/Central Africa were described as a genetically distinct group (Bertola *et al.* 2011a; Dubach *et al.* 2013; Barnett *et al.* 2014) (region definitions from IUCN SSC Cat Specialist Group 2006a; b, see Figure 1). The genetic dichotomy that separates the West/Central African lion populations from East and Southern African populations, has also been found in other large mammal species and is often reflected in their taxonomy, including African buffalo (*Syncerus caffer*) (Van Hooft *et al.* 2002; Smits *et al.* 2013), roan antelope (*Hippotragus equinus*) (Alpers *et al.* 2004), hartebeest (*Alcelaphus buselaphus*) (Arctander *et al.* 1999; Flagstad *et al.* 2001), giraffe (*Giraffa camelopardalis*) (Brown *et al.* 2007; Hassanin *et al.* 2007) and cheetah (*Acinonyx jubatus*) (Freeman *et al.* 2001; Charruau *et al.* 2011). However, mtDNA is a single, non recombining locus in the maternal lineage and does not permit the detection of admixture events and sorting at multiple loci, as may occur in autosomal markers. Therefore, the observed pattern in mtDNA data may not adequately depict the underlying genetic complexity.

Because the Asiatic lion subspecies occupies a nested position in the mtDNA based phylogenetic tree within the West/Central Africa clade, the current taxonomic division is challenged (Bertola *et al.* 2011a; Dubach *et al.* 2013; Barnett *et al.* 2014). The dichotomy within Africa has previously been described based on a range of morphometric data (Hemmer 1974) and the close relationship of West/Central African populations to the Asiatic subspecies is further supported by craniometric data (Mazák 2010). However, autosomal data are needed to assess how well the mtDNA tree represents the phylogeographic complexity in the lion, since conflicting patterns between phylogenies based on mtDNA and phylogenies based on autosomal markers have been described in several other species (Sota & Vogler 2001; DeBruyne 2005; Pinho *et al.* 2007; Roca *et al.* 2007; Zink & Barrowclough 2008; Rato *et al.* 2010). Most commonly a monophyletic pattern is detected in the mtDNA, but is not supported, or is contradicted, by phylogenies based on autosomal loci. This is often explained by incomplete lineage sorting, as coalescence time in mtDNA is four times shorter than in autosomal markers. Since lineage sorting during the process of coalescence has a random nature, this could also lead to an 'incorrect' gene tree by mtDNA markers if populations divergences were closely spaced in

time. Female philopatry is another strong contributing factor in mtDNA trees. As gene flow in lions is biased towards the male sex (Pusey *et al.* 1987; Spong *et al.* 2002), gene trees based on autosomal markers may show less discrete groups. This argument has been used by Antunes *et al.* (2008) to explain incongruent patterns in their lion data based on mtDNA and autosomal markers. Taxonomic revisions have potentially far-reaching ramifications with regard to management (e.g., CITES, USFWS, IUCN), and therefore, should be approached cautiously. Ideally proposed revisions should be supported by a combination of biogeographic, mtDNA and autosomal DNA, and morphological data.

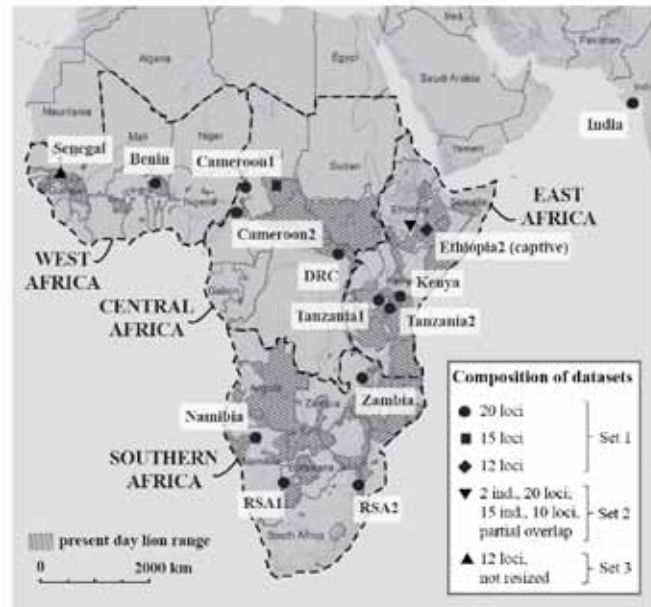
In this study, we analyzed 20 microsatellite loci for lions from thirteen wild populations, one of which is located in West Africa (Benin) and four in Central Africa (Chad, DRC and two from Cameroon). Furthermore, we included microsatellite data from another West African population in Senegal, and from two distinct zoo populations of Ethiopian lions. To compare the phylogenetic clusters derived from the microsatellite data and to check for congruence with previously published patterns, we included data from 1,454 base pairs (bp) of the mitochondrial DNA for each sampling location. Using this approach we are aiming to contribute in the ongoing discussion about lion taxonomy, by answering four questions: 1) Do autosomal data support previously described phylogenetic groupings in the lion in general and the distinct position of the West/Central African lion in particular? 2) Can an effect of sex-biased gene flow be detected? 3) How genetically distinct are the sampled populations, at both the continental and regional scales, and how do levels of genetic diversity compare amongst regional subdivisions? 4) Are there signs for reduced genetic diversity in particular lion populations with an emphasis on West/Central Africa? Our study is the first to include multiple lion populations from West/Central Africa, using both autosomal and mtDNA markers in a phylogenetic context covering the entire current geographic range of the lion.

## Materials and Methods

We processed a total of 48 samples from eight populations, including one population from West Africa (Benin), four populations from Central Africa (two from Cameroon, one from Chad and one from DRC), two populations from East Africa (Ethiopia2 (captive) and Kenya) and one population from Southern Africa (Zambia). Except for Ethiopia2, all included samples originated from free-ranging lions, with no known history of anthropogenic introductions of lions from other populations. Samples were collected in full compliance with specific permits (CITES and permits related to national legislation in the countries of origin). Details on permits, sample storage, DNA extraction, polymerase chain reaction (PCR) amplification, fragment analysis and quality control are given in Supplemental Information S1. See Supplemental Table S1 and S2 for used loci and primer information. All microsatellite allele length data are given in Supplemental Table S3.

Generated microsatellite data were supplemented by published data for the same 20 loci from another six populations (Driscoll *et al.* 2002), together summarized as Dataset 1. Dataset 2 consists of all 15 samples from Ethiopia1 (captive) with ten analyzed loci (Bruche *et al.* 2012), of which six are overlapping with our dataset. For two samples from Ethiopia1, all 20 microsatellites were analyzed and added to Dataset 1. Dataset 3 (*Panthera*/AMNH) contains microsatellite data from 12 loci for

seven lions from Senegal, which could not be resized to Dataset 1 and were therefore only included for calculation of diversity indices and bottleneck statistics (for details on permits and the processing of Senegal samples, see Supplemental Information S2). An overview of datasets used in each analysis is provided in Figure 1 and Table 1.



**Figure 1.** Map showing the location of the 16 lion populations included in the analysis. In the legend the composition of the datasets and the number of included microsatellite loci is indicated. Lion range data from IUCN (2014). Region definitions from IUCN SSC Cat Specialist Group (2006a; b).

**Table 1.** Overview of included lion populations in this study. PopSize: population size according to the most recent estimate in Riggio *et al.* (2012) for the African populations, except for Zambia: Paula White (personal communication); estimate for the Indian population from (Singh & Gibson 2011); N msat: number of sampled individuals for microsatellite analysis; N mtDNA: number of sampled individuals for mtDNA analysis.

| Set | Population | Area                | Geographic Region | PopSize   | N msat | N mtDNA | Source msat data              |
|-----|------------|---------------------|-------------------|-----------|--------|---------|-------------------------------|
| 1   | Benin      | Pendjari NP         | West Africa       | 100       | 5      | 5       | this dataset                  |
|     | Cameroon1  | Waza NP             | Central Africa    | 20        | 9      | 9       | this dataset                  |
|     | Cameroon2  | Bénoué Ecosystem    | Central Africa    | 200       | 3      | 3       | this dataset                  |
|     | Chad       | Zakouma NP          | Central Africa    | 140       | 4      | 4       | this dataset                  |
|     | DRC        | Garamba NP          | Central Africa    | 175       | 7      | 6       | this dataset                  |
|     | Ethiopia2  | Yemen Zoo           | East Africa       | (captive) | 4      | 4       | this dataset                  |
|     | Kenya      | Amboseli NP         | East Africa       | 60        | 7      | 7       | this dataset                  |
|     | Tanzania1  | Serengeti NP        | East Africa       | 3465      | 10     | 3       | Driscoll <i>et al.</i> , 2002 |
|     | Tanzania2  | Ngorongoro CA       | East Africa       | 53        | 10     | 1       | Driscoll <i>et al.</i> , 2002 |
|     | Zambia     | Luangwa Valley      | Southern Africa   | 750       | 9      | 9       | Driscoll <i>et al.</i> , 2002 |
| 2   | Namibia    | Etosha NP           | Southern Africa   | 455       | 10     | 2       | Driscoll <i>et al.</i> , 2002 |
|     | RSA1       | Kalahari-Gemsbok NP | Southern Africa   | 350       | 10     | 2       | Driscoll <i>et al.</i> , 2002 |
|     | RSA2       | Kruger NP           | Southern Africa   | 1684      | 10     | 10*     | Driscoll <i>et al.</i> , 2002 |
|     | India      | Gir forest NP       | India             | 411       | 10     | 6       | Driscoll <i>et al.</i> , 2002 |
|     | Ethiopia1  | Addis Ababa Zoo     | East Africa       | (captive) | 15     | 5       | Bruche <i>et al.</i> , 2012   |
| 3   | Senegal    | Niokolo Koba NP     | West Africa       | 15        | 7      | 7       | Panthera/AMNH                 |

\* mtDNA and microsatellite data are not from the same samples

STRUCTURE 2.3.3 (Pritchard *et al.* 2000) was used for assessing population structure in Dataset 1 with unknown loci scored as missing data. Simulations were run assuming the admixture model with correlated allele frequencies. Ten runs were performed for K=1 to K=11, using 1,000,000 permutations and a burn-in period of 100,000. To check the assignment of Ethiopia1 to any of the clusters identified by STRUCTURE, we included the two Ethiopian samples for all 20 microsatellites. The true value of K was determined using Structure Harvester (Evanno *et al.* 2005). CLUMPP was used to combine replicate runs and avoid label switching (Jakobsson & Rosenberg 2007). Clustering of individuals was further assessed by performing Principal Component Analysis (PCA) in GenAlEx 6.501 (Peakall & Smouse 2012). A neighbour-joining tree was created based on  $D_A$  distance in POPTREE2 using 1,000 bootstraps (Takezaki *et al.* 2010).

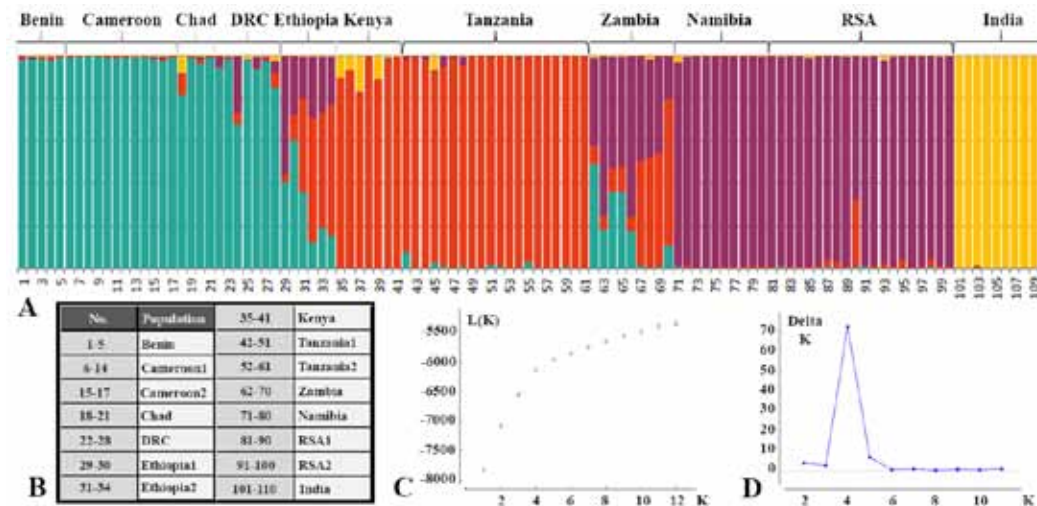
For each sampling location, a mitochondrial region of 1,454 bp that encompassed cytochrome b (cytB), tRNAThr, tRNAPro and part of the control region was included for a number of individuals (Table 1). Details on polymerase chain reaction (PCR) amplification and sequencing are given in Supplemental Information S1. Sequences were deposited in GenBank and supplemented by sequences previously published by Bertola *et al.* (2011) (see Supplemental Table S4 for accession numbers). Variable sites and nucleotide diversity were calculated using ARLEQUIN 3.5 (Excoffier *et al.* 2005). For phylogenetic analysis, a haplotype network was created using the median-joining algorithm in Network 4.6.1.1 (www.fluxus-engineering.com). A repeat region of cytosines of variable length was excluded due to unknown homology (bp 1382-1393) and all remaining characters were included with equal weighting.

For AMOVA of Dataset 1, individuals for which all 20 loci were analyzed were included as either 1) without an indicated substructure (as all 1 group), 2) following IUCN classification (Africa; Asia), 3) following a North/South division as was indicated from the haplotype network or 4) using the four groups identified by STRUCTURE (West/Central Africa; East Africa; Southern Africa; India). Isolation By Distance (IBD) was assessed by correlating geographic to genetic distances and using a Mantel's permutation test with 999 permutations, as implemented in GenAlEx 6.501 (Peakall & Smouse 2012). In addition, AMOVA and IBD analysis were performed on a regional level, using the regions as indicated above (Africa; North; South; West/Central Africa; East Africa; Southern Africa). Pairwise  $F_{ST}$  and Nei's genetic distances were computed with GenAlEx 6.501 (Peakall & Smouse 2012) for microsatellite data and with ARLEQUIN 3.5 for mtDNA data (Excoffier *et al.* 2005).

The average number of alleles per locus ( $N_a$ ) was calculated using ARLEQUIN 3.5 (Excoffier *et al.* 2005). Private allelic richness ( $N_{Pr}$ ) was calculated with HP-Rare 1.1 (Kalinowski 2005) including statistical rarefaction to compensate for different sample sizes. GenAlEx 6.501 (Peakall & Smouse 2012) was used to calculate observed ( $H_o$ ) and unbiased expected heterozygosity ( $uH_e$ ) (Nei 1978).  $F_{IS}$  per population was calculated in FSTAT (Goudet 2001). The occurrence of recent bottlenecks was evaluated by testing for heterozygosity excess using the program Bottleneck (Cornuet & Luikart 1996; Piry *et al.* 1999). The program was run for 10,000 iterations, using the stepwise mutation model (SMM). Significant (<0.05) results from the Wilcoxon signed-rank test were scored, as this test proved to be the most powerful and robust when used with few (<20) polymorphic loci (Piry *et al.* 1999).

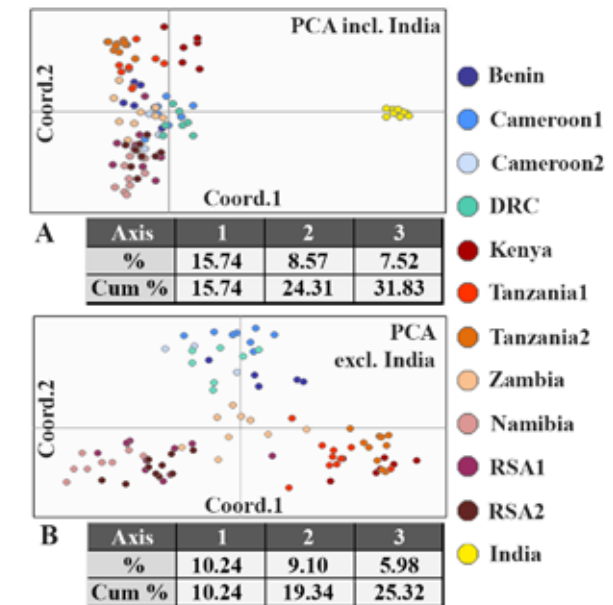
## Results

Based on the STRUCTURE results of Dataset 1, Structure Harvester identified that the observed genetic structure is best described by four clusters representing the following geographic areas: 1) West/Central Africa, 2) East Africa, 3) Southern Africa and 4) India (Figure 2). Individuals from Chad are part of the West/Central Africa cluster. The Ethiopian lions show affiliation either to West/Central Africa, admixed with Southern Africa (Ethiopia1) or to East Africa, admixed with Southern Africa (Ethiopia2). The Zambia population shows a substructure as a result of admixture. All Zambian individuals are partially assigned to the Southern Africa cluster, and depending on the individual, either to West/Central Africa, or to East Africa. The admixed signal of the Zambia population is also visible by the central position in the plot of the first two axes of the PCA when India is excluded (Figure 3B). Since PCA illustrated the effect of India (Figure 3A) and it is known that STRUCTURE may find fewer clusters than is expected based on known evolutionary history when one cluster is strongly deviating (Kalinowski 2010), STRUCTURE runs were repeated excluding Indian genotypes. These analyses did not lead to a difference in clustering of the remaining individuals and the same three groups were identified within Africa (data not shown).



**Figure 2.** Results of STRUCTURE analysis, based on 20 microsatellite loci of 15 lion populations (Dataset 1 + 2 individuals from Ethiopia1). A: representation of assignment values found by STRUCTURE, using K=4; B: overview of included populations; C: plot indicating mean log likelihood  $\ln(P(X|K))$ ; D: plot indicating DeltaK values as a function of the number of genetic clusters (K), in which  $\Delta K = \text{mean}(|L'(K)|)/\text{sd}(L(K))$ .

A total of 87 sequences of 1,454 bp were analyzed. Nucleotide diversity ( $\pi$ ) was 0.102. Based on 43 polymorphic sites, 15 different haplotypes were distinguished. The haplotype network (Figure 4A) and the neighbour-joining tree (Figure 4B) based on the microsatellite data show a similar topology, in which West/Central African lions are grouped together on a significantly supported branch (bootstrap value >70) and East and Southern African lions are represented on two different significantly supported branches (Figure 4B). A basal split into a North group (West/Central Africa and India) and a South group (East Africa and Southern Africa) is most clearly visible in the haplotype network, as the clustering of East Africa and Southern Africa on a South branch in the phenetic tree has only limited support. Furthermore, Kenya and India both have a basal and unresolved position in the tree.

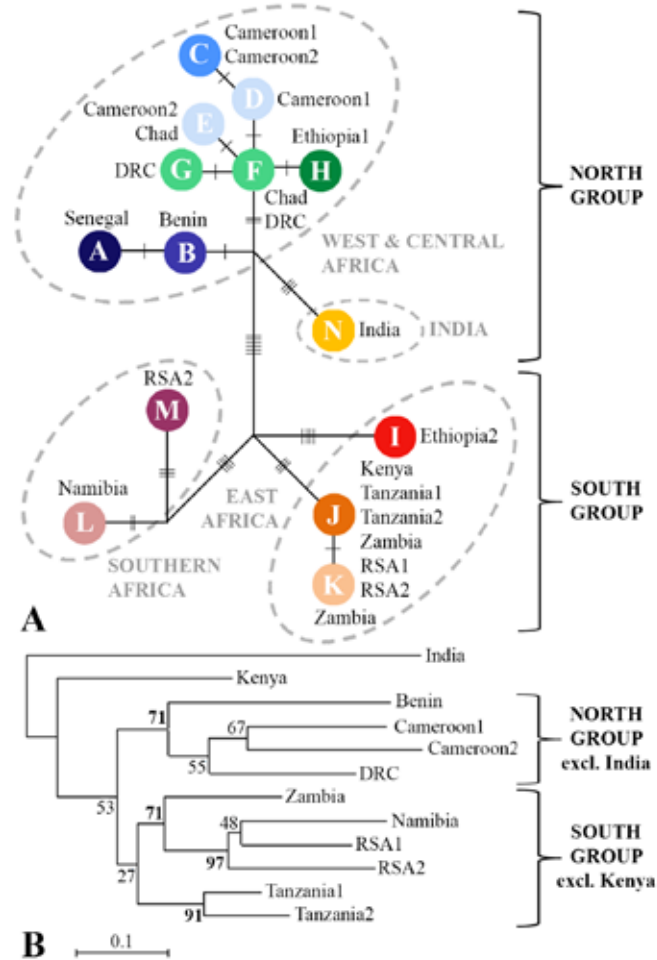


**Figure 3.** Results of PCA, based on 20 microsatellite loci of lion populations.

A: results PCA of 12 populations (Dataset 1, excluding Chad and Ethiopia2), shown in a two-dimensional plot and a table indicating the percentage and the cumulative percentage explained by the first three axes; B: results of PCA of 11 populations, excluding India.

Results from AMOVA of the microsatellite data show that following the clusters identified by STRUCTURE to assign populations to four groups, resulted in a relative high percentage of the molecular variance being attributed to among-groups for microsatellite data (17.4%) and mtDNA data (52.3%) (Supplemental Table S5). While in the microsatellite data the highest percentage (29.6%) of molecular variance in among-groups variance is attributed to the split between Africa and Asia, i.e. between the two subspecies, no molecular variance among-groups for the Africa/Asia division is found in the mtDNA data. In addition, following the basal split in a North group and a South group, AMOVA attributes 54.6% of molecular variance to among-groups variance for mtDNA data, but only finds 7.5% in among-groups variance when using microsatellite data. Absolute percentages may be misleading, as within-population variance is very different amongst the used markers.

Mantel tests showed that the effect of isolation by distance is evident, both on the continental and the regional scale (summary and graphs in Supplemental Table S6). In regional analyses, the highest values for among-groups variance according to AMOVA and the highest numbers for the slope of the trend line in IBD are found in West/Central Africa (compared to the South group, East Africa or Southern Africa) suggesting strong isolation between these populations. Pairwise  $F_{st}$  values ranged from 0.064 to 0.736 and were significant for all pairwise comparisons (50,000 permutations,  $P < 0.05$ ) (Supplemental Table S7). Within Africa pairwise  $F_{st}$  values ranged from 0.064 to 0.396. Nei's genetic distance ranged from 0.196 to 2.193 for all lion populations and within Africa it ranged from 0.196 to 2.018 (Supplemental Table S7).



**Figure 4.** Relationship between populations of lions based on mtDNA data and on 20 microsatellite loci. A: Haplotype network based on median-joining algorithm in Network; B: Phenetic tree based on Da genetic distance of microsatellite data of 12 lion populations.

Diversity indices (Supplemental Table S8) show that the Indian population comprises the lowest number of microsatellite alleles per locus, smallest allelic range and the highest number of fixed alleles. In the Indian population 75% of the loci are fixed while in all other populations at maximum 17% of the loci are fixed. Diversity indices were found to be relatively constant across the African populations; surprisingly West/Central Africa showed no clear signs of loss of genetic diversity. Four out of seven populations in West/Central Africa contained more than one haplotype (Cameroon1, Cameroon2, Chad, DRC), whereas this was only observed for two out of eight populations in East and Southern Africa (Zambia and RSA2). Observed and expected heterozygosity values further confirmed the low genetic diversity of the Indian population. Fis values illustrated a significant heterozygosity excess in Benin ( $P < 0.01$ ) and Cameroon1 ( $P < 0.01$ ) and a significant heterozygosity deficiency in Zambia ( $P < 0.01$ ), RSA1 ( $P < 0.05$ ) and Ethiopia1 ( $P < 0.05$ ). Results of the bottleneck analysis showed that there was a significant excess of heterozygotes found in Cameroon1 ( $P < 0.01$ ), Kenya ( $P < 0.05$ ) and Ethiopia1 ( $P < 0.05$ ), possibly indicating a recent reduction in population size.

## Discussion

Here we present an analysis of microsatellite and mtDNA datasets in lions sampled across their current geographic range. We included autosomal markers because this method had not been previously applied to investigate the genetic dichotomy between lion populations in West/Central Africa and those in East and Southern Africa. Moreover, we assessed levels of genetic diversity across different geographic scales to detect signs of low genetic diversity.

Analysis of microsatellite data (STRUCTURE) identified three clusters in the African lion: 1) West/Central Africa, 2) East Africa, and 3) Southern Africa, in addition to a cluster comprising the Asiatic subspecies. Although the high level of fixation of alleles in the Asiatic lion is likely to contribute to the identification of this population as a distinct cluster, genetic structure is found within the African subspecies. This supports the genetically distinct position of lions from West/Central Africa reported previously (Barnett *et al.* 2006b, 2014; Bertola *et al.* 2011a) and found again here based on mtDNA data. In addition, STRUCTURE also indicates divergence within the East and Southern African lions. The observed split between East and Southern Africa, as was previously shown by Bruche *et al.* (2012), remained after inclusion of a population from Zambia, geographically intermediate between Tanzania and RSA. Bruche *et al.* (2012) included lions from the Ethiopia1 population in a STRUCTURE analysis with data from Driscoll *et al.* (2002) and found a distinction between India, East Africa, Southern Africa and Ethiopia1. From this the authors concluded that the Ethiopia1 individuals form a unique group within the African lion. Including two individuals of Ethiopia1 in our microsatellite dataset, we find strong admixture with West/Central Africa. This is further confirmed by the mitochondrial haplotype of these lions, which is closely related to haplotypes found in Chad and DRC. The position of the Ethiopia1 lions in this study leads to the conclusion that these individuals do not form a unique group, but are instead assigned for a substantial part to a cluster that was not represented in the work by Bruche *et al.* (2012). Although the origin of the Ethiopia1 founder lions is disputed, it is claimed that they originate from the south-western part of Ethiopia (Tefera 2003), west of the Rift Valley, which has previously been suggested as a barrier for lion dispersal (Pitra *et al.* 2002; Burger *et al.* 2004; Dubach *et al.* 2005, 2013; Barnett *et al.* 2006b). The other captive Ethiopian population, Ethiopia2, contains a haplotype that clusters within the East Africa group. Assessment of the microsatellite data showed that Ethiopia2 individuals indeed contained a stronger signal from East Africa, compared to Ethiopia1. STRUCTURE analysis detected admixture in both captive Ethiopian lion populations that may be explained by the geographical location of Ethiopia. However, human-mediated translocations of lions between regions is not uncommon in zoo settings and may have contributed to the observed pattern. More data from free-ranging Ethiopian lions are required to determine if this pattern of admixture is accurately representative for that region. In Zambia, a substructure in the population is induced due to the two detected types of admixture: the Southern Africa cluster is admixed either with the West/Central Africa cluster, or with the East Africa cluster. These findings are parsimonious with the geographic isolation representative of Zambia's Luangwa Valley which is an offshoot of the Rift Valley System. We found no clear admixture between West/Central Africa with East Africa, possibly due to the Rift valley. The absence of a mitochondrial haplotype from outside the East Africa cluster in the Zambian individuals that were analyzed indicates that the pattern of admixture is likely due to male-mediated gene flow.

The mtDNA haplotype network shows the same four groups as identified in the STRUCTURE analysis: 1) West/Central Africa, 2) East Africa, 3) Southern Africa and 4) India. These groups have

also been proposed based on morphometric data, with a more basal clustering into a northern and a southern clade (Hemmer 1974). This coincides with the deepest split in the haplotype network which distinguishes a North group consisting of the West/Central African lion together with the Asiatic subspecies, and a South group consisting of lions from East and Southern Africa. Only a single or two closely related haplotypes are found in a single country, with two exceptions where more divergent haplotypes are present: 1) Ethiopia, which could be explained by the geographic location of the country as previously noted, and 2) RSA2, likely due to past translocations to and amongst small reserves in RSA (Miller *et al.* 2013). The geographical boundaries between the identified groups based on mtDNA and microsatellite data differ in the southern part of their range. STRUCTURE and PCA plots show that all populations from Namibia and RSA are assigned to Southern Africa, with a more central position for the admixed Zambia population, while East African haplotypes are found in RSA. The same discrepancy was previously described by Antunes *et al.* (2008) and attributed to sex-biased gene flow. The neighbour-joining tree, based on microsatellite data also shows a distinction between lions from West/Central Africa, and populations from East and Southern Africa. The basal position of the Indian and Kenyan lions probably results from the lower genetic diversity in these populations, as is indicated by the relatively high number of monomorphic loci. Elongation of branch length resulting from a population size reduction has been previously described, especially for DA as a measure of genetic distance (Kalinowski 2002). Despite of this DA is commonly accepted as the most suitable measure for inferring phylogenetic relationships, and therefore has been used in our analyses (Takezaki & Nei 1996, 2008). To further assess congruence between mtDNA and autosomal markers, a Mantel test was performed based on corrected Nei's genetic distances for both datasets (Supplemental Table S9). This illustrates a significant relationship (999 permutations,  $P < 0.01$ ) between both measures, which increases further after the exclusion of India.

Strongest congruence in AMOVA results between the autosomal and mtDNA data are found when using the groups identified by STRUCTURE. Microsatellite AMOVA show that the largest part of molecular variance is attributed to among-groups variance according to the IUCN classification, i.e. distinguishing an African and an Asiatic subspecies, which is also congruent with PCA results. This result is likely attributable to low genetic diversity and the high number of monomorphic loci in the Indian population rather than to long evolutionary distance. This is further confirmed by the fact that the Africa/Asia distinction leads to an exceptionally low percentage for among-groups variance when haplotype data are used. Haplotype data give the highest percentage for among-groups variance when following a distinction between a North group (West/Central Africa/India) and a South group (East Africa/Southern Africa), which is only moderately supported by microsatellite based among-group variance. Following the four groups identified by STRUCTURE shows a relative high among-group variance in both datasets, indicating a robust phylogenetic pattern that is reflected both in mtDNA and in autosomal DNA.

IBD explains the genetic distances on a continental scale and on a regional scale. The strong slope of the trend line in IBD analysis for West/Central Africa, compared to Southern and East Africa, is suggestive of near complete isolation between populations in the West/Central region. This is also supported by the high among-groups variance in the AMOVA. Based on the genetic distances (pairwise  $F_{st}$  and Nei's genetic distance), we conclude that all sampled populations are significantly differentiated from each other.

It was hypothesized that lion populations in West Africa and parts of Central Africa were especially

vulnerable to declining levels of genetic diversity since fragmentation of the habitat is particularly severe in this region. However, we did not find significant heterozygotic deficiencies, reduced number of alleles or fixed loci in any of the six sampled populations in this region. The significantly negative  $F_{is}$  values (excess of heterozygotes) may be explained by the mating system as was also shown for prides in Selous GR (Spong *et al.* 2002), however we acknowledge the possible effect of a small sample size in our study. The strongly significant heterozygote deficiency observed in the Zambia lion population is likely to be the result of substructure in the population (Wahlund effect), which was consistent with the results from the STRUCTURE analysis. The significantly positive  $F_{is}$  value found in RSA1 is congruent with previous findings (Dubach *et al.* 2013): Dubach *et al.* (2013) reports a relative high value for the Kalahari population (RSA1), although it was tested as non significant. A high  $F_{is}$  value in the Ethiopia1 lions can be explained by the breeding history of the population, which was founded by five males and two females in 1948 (Bruche *et al.* 2012). Bottleneck analysis indicated that both RSA1 and Ethiopia1 have gone through recent population reductions. Similarly, Cameroon1 and Kenya appear to have experienced bottlenecks, which is consistent with observations obtained from monitoring studies (Iongh *et al.* 2009; Riggio *et al.* 2012). Since the excess of heterozygotes as a result of a bottleneck is transient, the Bottleneck approach only detects recent reductions in population size, which explains why historically documented bottlenecks i.e., Tanzania2 and India, were not detected.

The unexpected high levels of genetic diversity found in West/Central Africa lions could be explained by the fact that the range contraction and the decline of lion numbers is too recent to show clear signs of genetic erosion at this point. However, as genetic diversity is rapidly lost in small populations as a result of genetic drift and inbreeding, keeping the population at a genetically healthy level may require urgent management decisions to safeguard against these effects. Monitoring of an intensively managed lion population showed that drift and inbreeding were noticeable within five years after reintroduction of eleven founders from four genetic lineages (Trinkel *et al.* 2010). By showing a congruent phylogeographic pattern in both mtDNA and autosomal markers, our data illustrate which populations belong to the same evolutionary lineage and may contribute importantly to conservation decisions e.g., identifying suitable candidates for translocations or population augmentation.

Our study is the first to confirm that autosomal markers support the distinct genetic position of West/Central African lions within the African subspecies. The phylogenetic split between West/Central Africa and East/Southern Africa found in other species is reiterated in lions. Based on results derived from mtDNA data and from autosomal microsatellites, we recommend recognition and consideration of these four groups for management decisions: 1) West/Central Africa, 2) East Africa, 3) Southern Africa and 4) India. In consideration of genetic distinctions coupled with anthropogenic factors that are accelerating decline of wildlife in West and Central Africa, this region is of particular and urgent conservation importance. We support a revision of the taxonomic nomenclature as has been proposed by Barnett *et al.* (2014), following the deepest ancestral split found in the haplotype network, recognizing a North group and a South group. In addition, there may be arguments to warrant the Asiatic population its subspecies status (Dubach *et al.* 2013), although this would lead to a paraphyletic status of (one of) the other subspecies due to the close genetic relationship between lions from West/Central Africa and India. Primarily, as mtDNA, autosomal markers and morphological data show a congruent pattern, we believe that it is enough support for a taxonomic split within the African subspecies of the lion.

## Acknowledgements

Samples were kindly provided by N. Vanherle (CURESS, Chad), S. Adam and selected members of the West/Central African lion network (ROCAL), R. Buij and B. Croes (Cameroon), ICCN, Garamba NP (DRC), South Africa National Parks (SANParks) (RSA2). We also thank Direction des Parcs Nationaux (DPN - Senegal) for facilitating sample collection and export. Further, we thank F. Oberwemmer (Leipzig Zoo) and IZW Leibnitz Institute for Zoo and Wildlife Research (Ethiopia1: Addis Ababa Zoo), and Sanaa Zoo, Yemen (Ethiopia2) for access to samples of two captive populations. We are grateful for the permit of the Ethiopian Wildlife Conservation Authority (formerly Wildlife Conservation Department) to obtain and export blood samples of the lions from Addis Ababa Zoo (Ethiopia1) through Zoo Leipzig, Germany. We would like to thank S. Bruche and coauthors for enabling us to include their data (Dataset 2) and S. Rabinowitz and S. Loss (American Museum of Natural History) for their contribution to the Senegal data (Dataset 3). We further thank H. Visser for contributing to processing of the samples. The investigations were supported by the Division for Earth and Life Sciences (ALW) with financial aid from the Netherlands Organization for Scientific Research (NWO) (project no. 820.01.002).

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### Data accessibility

Microsatellite data are accessible in Supplemental Table S3. All sequence data generated in this study have been submitted to GenBank. Accession numbers are listed in Supplemental Table S4.

### 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. Overview of microsatellite loci used in the different lion populations. No. loci: number of included loci.

| Set | Population | No. loci | Microsatellite loci                                                                                                                                            |
|-----|------------|----------|----------------------------------------------------------------------------------------------------------------------------------------------------------------|
| 1   | Benin      | 20       | FCA026, FCA032, FCA057, FCA075, FCA085, FCA091, FCA094, FCA097, FCA126, FCA136, FCA144, FCA161, FCA178, FCA191, FCA205, FCA208, FCA211, FCA224, FCA247, FCA275 |
|     | Cameroon1  | 20       | FCA026, FCA032, FCA057, FCA075, FCA085, FCA091, FCA094, FCA097, FCA126, FCA136, FCA144, FCA161, FCA178, FCA191, FCA205, FCA208, FCA211, FCA224, FCA247, FCA275 |
|     | Cameroon2  | 20       | FCA026, FCA032, FCA057, FCA075, FCA085, FCA091, FCA094, FCA097, FCA126, FCA136, FCA144, FCA161, FCA178, FCA191, FCA205, FCA208, FCA211, FCA224, FCA247, FCA275 |
|     | Chad       | 15       | FCA032, FCA057, FCA075, FCA091, FCA094, FCA097, FCA126, FCA136, FCA144, FCA161, FCA205, FCA208, FCA224, FCA247, FCA275                                         |
|     | DRC        | 20       | FCA026, FCA032, FCA057, FCA075, FCA085, FCA091, FCA094, FCA097, FCA126, FCA136, FCA144, FCA161, FCA178, FCA191, FCA205, FCA208, FCA211, FCA224, FCA247, FCA275 |
|     | Ethiopia2  | 12       | FCA032, FCA075, FCA091, FCA094, FCA097, FCA126, FCA144, FCA205, FCA208, FCA224, FCA247, FCA275                                                                 |
|     | Kenya      | 20       | FCA026, FCA032, FCA057, FCA075, FCA085, FCA091, FCA094, FCA097, FCA126, FCA136, FCA144, FCA161, FCA178, FCA191, FCA205, FCA208, FCA211, FCA224, FCA247, FCA275 |
|     | Tanzania1  | 20       | FCA026, FCA032, FCA057, FCA075, FCA085, FCA091, FCA094, FCA097, FCA126, FCA136, FCA144, FCA161, FCA178, FCA191, FCA205, FCA208, FCA211, FCA224, FCA247, FCA275 |
|     | Tanzania2  | 20       | FCA026, FCA032, FCA057, FCA075, FCA085, FCA091, FCA094, FCA097, FCA126, FCA136, FCA144, FCA161, FCA178, FCA191, FCA205, FCA208, FCA211, FCA224, FCA247, FCA275 |
|     | Zambia     | 20       | FCA026, FCA032, FCA057, FCA075, FCA085, FCA091, FCA094, FCA097, FCA126, FCA136, FCA144, FCA161, FCA178, FCA191, FCA205, FCA208, FCA211, FCA224, FCA247, FCA275 |
|     | Namibia    | 20       | FCA026, FCA032, FCA057, FCA075, FCA085, FCA091, FCA094, FCA097, FCA126, FCA136, FCA144, FCA161, FCA178, FCA191, FCA205, FCA208, FCA211, FCA224, FCA247, FCA275 |
|     | RSA1       | 20       | FCA026, FCA032, FCA057, FCA075, FCA085, FCA091, FCA094, FCA097, FCA126, FCA136, FCA144, FCA161, FCA178, FCA191, FCA205, FCA208, FCA211, FCA224, FCA247, FCA275 |
|     | RSA2       | 20       | FCA026, FCA032, FCA057, FCA075, FCA085, FCA091, FCA094, FCA097, FCA126, FCA136, FCA144, FCA161, FCA178, FCA191, FCA205, FCA208, FCA211, FCA224, FCA247, FCA275 |
|     | India      | 20       | FCA026, FCA032, FCA057, FCA075, FCA085, FCA091, FCA094, FCA097, FCA126, FCA136, FCA144, FCA161, FCA178, FCA191, FCA205, FCA208, FCA211, FCA224, FCA247, FCA275 |
| 2   | Ethiopia1  | 10*      | FCA006, FCA082, FCA097, FCA136, FCA161, FCA178, FCA191, FCA200, FCA211, FCA249                                                                                 |
|     | Senegal    | 12**     | FCA032, FCA075, FCA096, FCA100, FCA124, FCA126, FCA132, FCA208, FCA212, FCA225, FCA229, FCA275                                                                 |

\* for two samples all twenty loci were analysed, for the others ten loci were analyzed of which six overlap with Dataset 1  
\*\* not resized

Supplemental Table S2. Primers used for amplification of microsatellites and mtDNA.  
Location: targeted genetic marker, for microsatellite loci the chromosomal location in cat (Felis catus) is indicated; Annealing Temp: annealing temperature in °C.

| Type           | Location                                                                         | Primer name     | Annealing Temp (°C) | Sequence (5'-3')          | Origin                       |
|----------------|----------------------------------------------------------------------------------|-----------------|---------------------|---------------------------|------------------------------|
| mtDNA          | Cytochrome b, tRNA <sup>Pro</sup> , tRNA <sup>Thr</sup> , partial control region | F: 1F           | 51                  | CGTTGTACTTCAACTATAAGAACTT | own design                   |
|                |                                                                                  | R: 1R           |                     | ATGGGATTGCTGATAGGAGATTAG  | own design                   |
|                |                                                                                  | F: 2F           | 53                  | GTGGGGCCAAATATCTTTT       | own design                   |
|                |                                                                                  | R: 2R internal* |                     | GAAGGCCTAGGATATCTTTGATTG  | own design                   |
|                |                                                                                  | F: 3F internal* | 51                  | GACTCAGATAAAATCCATTCCA    | own design                   |
|                |                                                                                  | R: 3R internal* |                     | CATTATCTCGCTGTTTGG        | own design                   |
|                |                                                                                  | F: 4F internal* | 51                  | CAATTATCCCTGCCCTCCA       | own design                   |
|                |                                                                                  | R: 4R           |                     | TTTTTGTTTACAAGACCAAGGTA   | own design                   |
|                |                                                                                  | F: 5F           | 54                  | AAATCGCCTCTCAAATGAA       | own design                   |
|                |                                                                                  | R: 5R           |                     | AATATTCATGGGAGGGCAGTC     | own design                   |
|                |                                                                                  | F: FCA026F      | 51                  | GGAGCCCTTAGAGCTATGCA      | Menotti-Raymond et al., 1999 |
|                |                                                                                  | R: FCA026R      |                     | TGTACACGCACCAAAACAA       | Menotti-Raymond et al., 1999 |
| Microsatellite | Chromosome A2 (Cat)                                                              | F: FCA032F      | 51                  | GGCAATTCATGGTAGAGAAAA     | Menotti-Raymond et al., 1999 |
|                |                                                                                  | R: FCA032R      |                     | CAAGAGTCATTGGGCAGTA       | Menotti-Raymond et al., 1999 |
|                | Chromosome C1 (Cat)                                                              | F: FCA057F      | 51                  | AAGTGTGGGATTGGGTGAAA      | Menotti-Raymond et al., 1999 |
|                |                                                                                  | R:FCA057R       |                     | CCATAAGAGGCTCTTAAAACTGA   | Menotti-Raymond et al., 1999 |
|                | Chromosome A2 (Cat)                                                              | F: FCA075F      | 51                  | ATGCTAATCAGTGGCATTTGG     | Menotti-Raymond et al., 1999 |
|                |                                                                                  | R:FCA075R       |                     | GAACAAAAATCCAGACGTGC      | Menotti-Raymond et al., 1999 |
|                | Chromosome A2 (Cat)                                                              | F: FCA085F      | 51                  | CTGTACATTTCTCTCCCATTCG    | Menotti-Raymond et al., 1999 |
|                |                                                                                  | R: FCA085R      |                     | CCCCTACTGGGTGCACGTG       | Menotti-Raymond et al., 1999 |
|                | Chromosome B4 (Cat)                                                              | F: FCA091F      | 51                  | TGAGAACAAGCCATTAAATAGCA   | Menotti-Raymond et al., 1999 |
|                |                                                                                  | R: FCA091R      |                     | CCCAACATAAGGCTGCATT       | Menotti-Raymond et al., 1999 |
|                | Chromosome F2 (Cat)                                                              | F: FCA094F      | 51                  | TCAAGCCCCATTTTACCTTC      | Menotti-Raymond et al., 1999 |
|                |                                                                                  | R: FCA094R      |                     | CACCTGAGCCAAAGGCTATC      | Menotti-Raymond et al., 1999 |
|                | Chromosome B1 (Cat)                                                              | F: FCA097F      | 51                  | TAATGTTCAACTGAATTGCTTCC   | Menotti-Raymond et al., 1999 |
|                |                                                                                  | R: FCA097R      |                     | GAACAGTAGTTTGCCCATACAGG   | Menotti-Raymond et al., 1999 |
|                | Chromosome B1 (Cat)                                                              | F: FCA126F      | 51                  | GCCCCTGATACCTGAATG        | Menotti-Raymond et al., 1999 |
|                |                                                                                  | R: FCA126R      |                     | CTATCCTTGCTGGCTGAAGG      | Menotti-Raymond et al., 1999 |
|                | Chromosome F2 (Cat)                                                              | F: FCA136F      | 51                  | GAATGACATCGCCAATGAAA      | Menotti-Raymond et al., 1999 |
|                |                                                                                  | R: FCA136R      |                     | CCCCCCCCAACTGATACTT       | Menotti-Raymond et al., 1999 |
|                | Chromosome D1 (Cat)                                                              | F: FCA144F      | 51                  | GGAATCTCGTGAACCTCTGTC     | Menotti-Raymond et al., 1999 |
|                |                                                                                  | R: FCA144R      |                     | CCCGGCAAAATTATGAAGG       | Menotti-Raymond et al., 1999 |
|                | Chromosome A3 (Cat)                                                              | F: FCA161F      | 51                  | TTACCGATACACACCTGCCA      | Menotti-Raymond et al., 1999 |
|                |                                                                                  | R: FCA161R      |                     | CACAGACGTGCTCTAGCCAA      | Menotti-Raymond et al., 1999 |
|                | Chromosome A1 (Cat)                                                              | F: FCA178F      | 51                  | GTGCCCCATGAATCTCACTT      | Menotti-Raymond et al., 1999 |
|                |                                                                                  | R: FCA178R      |                     | TACAACTCAGGGGTCGTATGG     | Menotti-Raymond et al., 1999 |
|                | Chromosome C1 (Cat)                                                              | F: FCA191F      | 51                  | TCCTGTTCCTATTCACCTTACA    | Menotti-Raymond et al., 1999 |
|                |                                                                                  | R: FCA191R      |                     | GCATGGCACTTTTGTGAGA       | Menotti-Raymond et al., 1999 |
|                | Chromosome B3 (Cat)                                                              | F: FCA205F      | 51                  | CCTGCTCTCAAGGAGCTCC       | Menotti-Raymond et al., 1999 |
|                |                                                                                  | R: FCA205R      |                     | CCCATTCTCCTACCACTTCC      | Menotti-Raymond et al., 1999 |
|                | Chromosome A3 (Cat)                                                              | F: FCA208F      | 51                  | TCAGGGTTCAAAAAAGAAAAA     | Menotti-Raymond et al., 1999 |
|                |                                                                                  | R: FCA208R      |                     | CAAAGCACCAGCTTAGAAGTCA    | Menotti-Raymond et al., 1999 |
|                | Chromosome B1 (Cat)                                                              | F: FCA211F      | 51                  | TGTAGAACATAATGCCTCAGCC    | Menotti-Raymond et al., 1999 |
|                |                                                                                  | R: FCA211R      |                     | TCTTGAACCTATTTCCTCACA     | Menotti-Raymond et al., 1999 |
|                | Chromosome A3 (Cat)                                                              | F: FCA224F      | 51                  | CTGGGTGCTGACAGCATAGA      | Menotti-Raymond et al., 1999 |
|                |                                                                                  | R: FCA224R      |                     | TGCCAGAGTTGTATGAAAGGG     | Menotti-Raymond et al., 1999 |
|                | Chromosome C1 (Cat)                                                              | F: FCA247F      | 51                  | GGAAATTAGGAGCTCTGCCA      | Menotti-Raymond et al., 1999 |
|                |                                                                                  | R: FCA247R      |                     | AAGATTTACCCAGTTGCCCC      | Menotti-Raymond et al., 1999 |
|                | Chromosome B2 (Cat)                                                              | F: FCA275F      | 51                  | TTGGCTGCCCACTTTTATGTT     | Menotti-Raymond et al., 1999 |
|                |                                                                                  | R: FCA275R      |                     | ACGAAGGGGCAGGACTATCT      | Menotti-Raymond et al., 1999 |

\* internal primers were only used when amplification with primerpair 2F-4R failed

**Supplemental Table S3. (online only)** Microsatellite data for 20 loci in 16 lion populations.

**Supplemental Table S4.** Identified haplotypes and accompanying accession numbers from Genbank.

| Haplotype | Country                                              | Genbank Accession                        | Source               |
|-----------|------------------------------------------------------|------------------------------------------|----------------------|
| A         | Senegal                                              | KJ652247                                 | this publication     |
| B         | Benin                                                | GU131164 - GU131165                      | this publication     |
| C         | Cameroon1 + Cameroon2                                | GU131174 - GU131175, AY781202 - AY781205 | Bertola et al., 2011 |
| D         | Cameroon1                                            | GU131170 - GU131173                      | Bertola et al., 2011 |
| E         | Cameroon2 + Chad                                     | GU131169, AY781198 - AY781199, AY781197  | Bertola et al., 2011 |
| F         | Chad+DRC                                             | AY781200, DQ018993 - DQ018994            | this publication     |
| G         | DRC                                                  | KJ652248                                 | this publication     |
| H         | Ethiopia1                                            | KJ652249                                 | this publication     |
| I         | Ethiopia2                                            | AY781207 - AY781210                      | Bertola et al., 2011 |
| J         | Kenya + Tanzania1 + Tanzania2 + Zambia + RSA1 + RSA2 | GU131166 - GU131168                      | this publication     |
| K         | Zambia                                               | KJ652250                                 | this publication     |
| L         | Namibia                                              | KJ652251                                 | this publication     |
| M         | RSA2                                                 | GU131183 - GU131185                      | this publication     |
| N         | India                                                | GU131176 - GU131178, AY781206            | this publication     |

Unique point mutations (i.e. observed in a single sample) were checked by an independent PCR and sequencing. This resulted in correcting the following previously published sequences by Bertola et al. (2011) on Genbank (September 2013): GU131167-GU131168, GU131170, GU131172-GU131175, GU131178, GU131183, GU131185. Conclusions based on the uncorrected sequences as published in Bertola et al. (2011) still hold.

**Supplemental Table S5.** Results of an AMOVA for a microsatellite dataset of 12 lion populations and a mtDNA dataset of 16 lion populations.

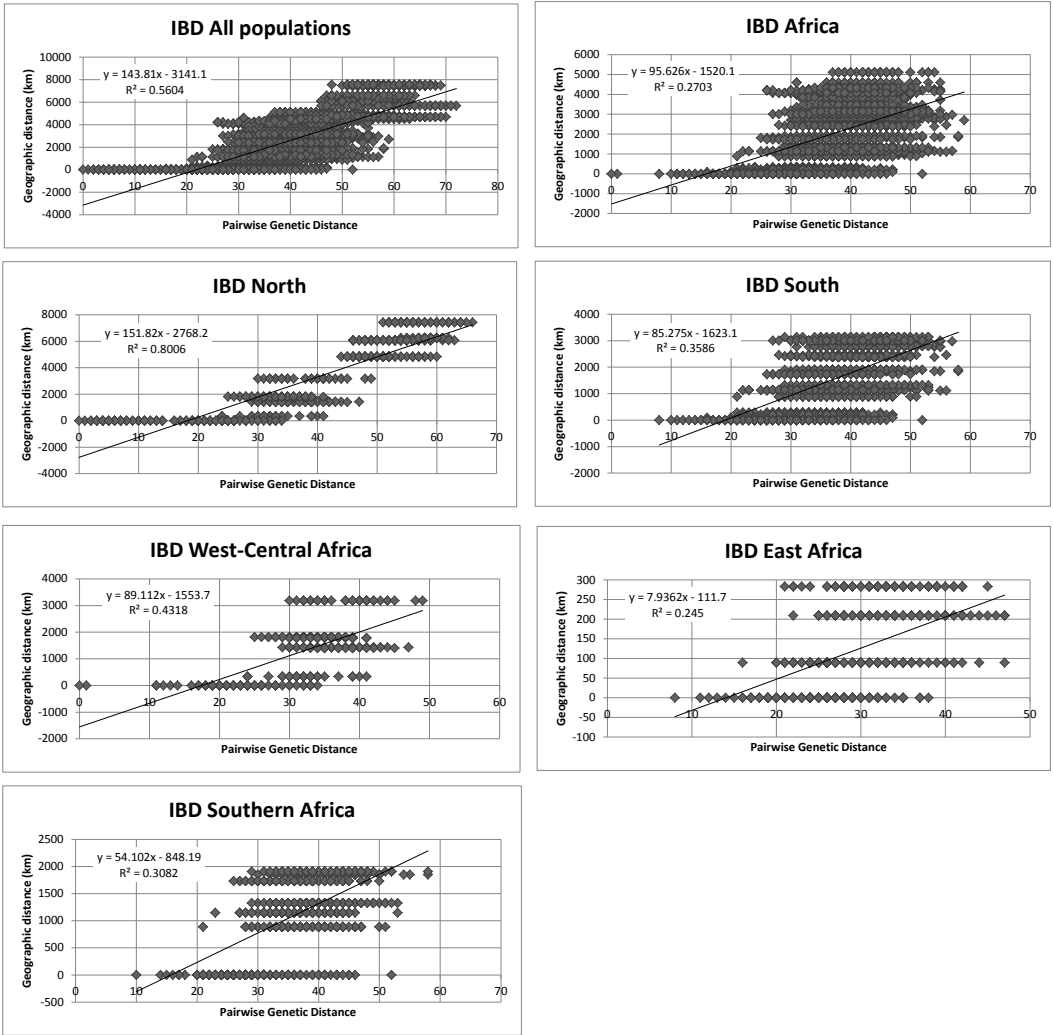
Four different divisions were tested: no substructure (all in one group), following IUCN categorisation (2 groups), following haplotype structure (2 groups) and following STRUCTURE results (4 groups); Variance component AG: Among Groups; AP: Among Populations; AI: Among Individuals; Within Groups; WP: Within Populations; WI: Within Individuals; df: degrees of freedom; % Total: percentage of total molecular variance explained on the different levels

| Number of groups | Division                                                            | Variance component | microsatellites |                |                        |         | mtDNA |                |                        |         |
|------------------|---------------------------------------------------------------------|--------------------|-----------------|----------------|------------------------|---------|-------|----------------|------------------------|---------|
|                  |                                                                     |                    | df              | Sum of squares | Variants of components | % Total | df    | Sum of squares | Variants of components | % Total |
| 1                | No division                                                         | AP                 | 11              | 494.445        | 2.37882                | 29.9    | 15    | 406.281        | 5.29171                | 98.1    |
|                  |                                                                     | AI/WP              | 88              | 489.720        | -0.0225                | -0.3    | 67    | 6.972          | 0.10406                | 1.9     |
|                  |                                                                     | WI                 | 100             | 561.000        | 5.61000                | 70.4    | -     | -              | -                      | -       |
| 2                | Africa; Asia                                                        | AG                 | 1               | 151.848        | 3.09325                | 29.6    | 1     | 32552          | 0.04718                | 0.9     |
|                  |                                                                     | AP/WG              | 10              | 342.597        | 1.76592                | 16.9    | 14    | 373729         | 5.28484                | 97.2    |
|                  |                                                                     | WP                 | 188             | 1050.720       | 5.58894                | 53.5    | 67    | 6972           | 0.10406                | 1.9     |
| 2                | North (West/Central Africa + India); South (East & Southern Africa) | AG                 | 1               | 96.294         | 0.62456                | 7.5     | 1     | 183037         | 3.93253                | 54.6    |
|                  |                                                                     | AP/WG              | 10              | 398.151        | 2.06955                | 25.0    | 14    | 223244         | 3.17305                | 44.0    |
|                  |                                                                     | WP                 | 188             | 1050.720       | 5.58894                | 67.5    | 67    | 6972           | 0.10406                | 1.4     |
| 4                | West/Central Africa; East Africa; Southern Africa; India            | AG                 | 3               | 289.637        | 1.43925                | 17.4    | 3     | 237368         | 3.3016                 | 52.3    |
|                  |                                                                     | AP/WG              | 8               | 204.808        | 1.25075                | 15.1    | 12    | 168913         | 2.91068                | 46.1    |
|                  |                                                                     | WP                 | 188             | 1050.720       | 5.58894                | 67.5    | 67    | 6972           | 0.10406                | 1.7     |

**Supplemental Table S6.** Results of the Mantel tests indicating IBD effects in lion populations on continental and regional scale.

NPop: number of included populations; Pair. comparisons: number of pairwise comparisons; Rxy: correlation coefficient (P(Rxy\_random>Rxy\_data), one-tailed, 999 permutations); x: slope of regression line (95% confidence interval (CI)); AG: Among group variance according to regional AMOVA.

| Groups                               | NPop | Pair. comparisons | Rxy             | x (95% CI)             | AG    |
|--------------------------------------|------|-------------------|-----------------|------------------------|-------|
| All populations                      | 12   | 4950              | 0.749 (P≤0.001) | 143.81 (140.26-147.36) | 29.86 |
| Africa                               | 11   | 4005              | 0.520 (P≤0.001) | 95.63 (90.76-100.50)   | 22.10 |
| North (West/Central Africa; India)   | 5    | 561               | 0.895 (P≤0.001) | 151.82 (145.53-158.12) | 42.18 |
| South (East Africa; Southern Africa) | 7    | 2145              | 0.599 (P≤0.001) | 85.28 (80.44-90.11)    | 19.47 |
| West/Central Africa                  | 4    | 276               | 0.657 (P≤0.001) | 89.11 (76.95-101.27)   | 20.48 |
| East Africa                          | 3    | 351               | 0.495 (P≤0.001) | 7.94 (6.47-9.40)       | 13.77 |
| Southern Africa                      | 4    | 741               | 0.555 (P≤0.001) | 54.1 (48.25-59.96)     | 15.18 |



**Supplemental Table S7.** Pairwise Fst (below diagonal) and Nei’s genetic distances (above diagonal) based on 20 microsatellite loci from 14 lion populations.

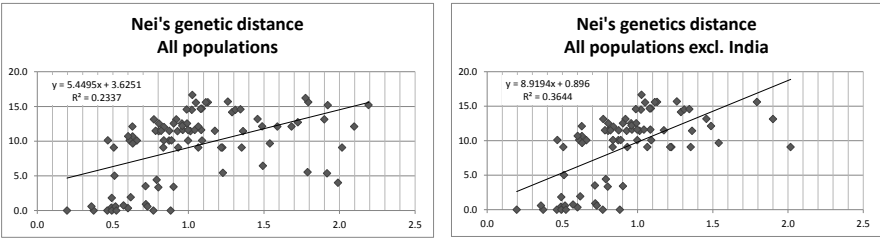
|           | Benin | Cameroon1 | Cameroon2 | Chad  | DRC   | Ethiopia2 | Kenya | Tanzania1 | Tanzania2 | Zambia | Namibia | RSA1  | RSA2  | India |
|-----------|-------|-----------|-----------|-------|-------|-----------|-------|-----------|-----------|--------|---------|-------|-------|-------|
| Benin     | -     | 0.789     | 0.801     | 0.716 | 0.902 | 1.900     | 1.091 | 0.872     | 0.659     | 0.630  | 0.920   | 0.997 | 1.289 | 1.990 |
| Cameroon1 | 0.242 | -         | 0.491     | 0.619 | 0.493 | 1.794     | 0.957 | 0.805     | 0.903     | 0.772  | 1.129   | 0.991 | 1.027 | 1.493 |
| Cameroon2 | 0.268 | 0.134     | -         | 0.600 | 0.571 | 1.311     | 1.362 | 0.927     | 1.014     | 0.839  | 1.022   | 0.804 | 1.050 | 1.918 |
| Chad      | 0.169 | 0.160     | 0.104     | -     | 0.718 | 1.089     | 1.085 | 1.042     | 0.963     | 0.959  | 1.081   | 0.998 | 1.261 | 1.789 |
| DRC       | 0.225 | 0.147     | 0.140     | 0.146 | -     | 1.344     | 1.175 | 0.783     | 0.820     | 0.629  | 0.989   | 0.874 | 1.115 | 1.228 |
| Ethiopia2 | 0.396 | 0.337     | 0.301     | 0.269 | 0.239 | -         | 1.225 | 1.353     | 2.018     | 1.539  | 1.488   | 1.217 | 1.458 | 2.193 |
| Kenya     | 0.336 | 0.337     | 0.348     | 0.267 | 0.264 | 0.296     | -     | 0.373     | 0.463     | 0.729  | 1.065   | 0.882 | 0.837 | 1.065 |
| Tanzania1 | 0.265 | 0.264     | 0.247     | 0.218 | 0.170 | 0.263     | 0.163 | -         | 0.196     | 0.358  | 0.834   | 0.493 | 0.612 | 1.589 |
| Tanzania2 | 0.271 | 0.321     | 0.318     | 0.260 | 0.231 | 0.391     | 0.229 | 0.064     | -         | 0.518  | 0.932   | 0.768 | 0.888 | 2.098 |
| Zambia    | 0.189 | 0.225     | 0.225     | 0.189 | 0.134 | 0.267     | 0.234 | 0.087     | 0.183     | -      | 0.631   | 0.523 | 0.601 | 1.724 |
| Namibia   | 0.289 | 0.300     | 0.259     | 0.248 | 0.218 | 0.307     | 0.334 | 0.245     | 0.304     | 0.206  | -       | 0.506 | 0.511 | 1.922 |
| RSA1      | 0.253 | 0.262     | 0.211     | 0.180 | 0.188 | 0.210     | 0.246 | 0.133     | 0.234     | 0.138  | 0.156   | -     | 0.467 | 1.683 |
| RSA2      | 0.268 | 0.262     | 0.214     | 0.228 | 0.198 | 0.246     | 0.258 | 0.170     | 0.253     | 0.139  | 0.166   | 0.118 | -     | 1.775 |
| India     | 0.699 | 0.602     | 0.720     | 0.661 | 0.552 | 0.736     | 0.589 | 0.577     | 0.643     | 0.575  | 0.601   | 0.551 | 0.562 | -     |

**Supplemental Table S8.** Genetic variation in microsatellite loci and mtDNA among 16 lion populations. No.loci: number of amplified loci; Np: number of polymorphic loci (% of total amplified loci); Na: average number of alleles per locus; Allelic range: average over allelic ranges calculated as number of repeats per locus; NaPr: private allelic richness, calculated over the complete dataset; Haplo.: haplotypes detected in this population, as referred to in Figure 4; Ho: observed heterozygosity; uHe: unbiased expected heterozygosity; Fis: Fis index, P indicated between brackets for samples which tested significant; Bottlen.: indicated are significant results from the Wilcoxon signed-rank test in the program Bottleneck. Standard errors (SE) are presented in parentheses.

| Set | Population | No. loci | Np (%)     | Na (±SE)     | Allelic range (±SE) | NaPr | Haplo. | Ho (±SE)     | uHe (±SE)    | Fis (P)         | Bottlen. |
|-----|------------|----------|------------|--------------|---------------------|------|--------|--------------|--------------|-----------------|----------|
| 1   | Benin      | 20       | 19 (95%)   | 2.95 (±1.00) | 5.10 (±4.81)        | 0.05 | B      | 0.65 (±0.07) | 0.55 (±0.05) | -0.204 (P<0.01) |          |
|     | Cameroon1  | 20       | 20 (100%)  | 3.20 (±0.77) | 4.65 (±3.82)        | 0.00 | C+D    | 0.68 (±0.05) | 0.61 (±0.02) | -0.129 (P<0.01) | P<0.05   |
|     | Cameroon2  | 20       | 19 (95%)   | 2.85 (±0.88) | 4.75 (±3.25)        | 0.05 | C+E    | 0.58 (±0.07) | 0.61 (±0.05) | 0.060           |          |
|     | Chad       | 15       | 15 (100%)  | 2.60 (±1.82) | 7.40 (±5.77)        | 0.20 | E+F    | 0.6 (±0.07)  | 0.56 (±0.05) | 0.085           |          |
|     | DRC        | 20       | 20 (100%)  | 4.65 (±1.87) | 6.40 (±6.37)        | 0.10 | F+G    | 0.74 (±0.04) | 0.7 (±0.03)  | -0.066          |          |
|     | Ethiopia2  | 12       | 10 (83.3%) | 1.25 (±1.16) | 3.33 (±3.62)        | 0.00 | I      | 0.44 (±0.1)  | 0.41 (±0.07) | -0.068          |          |
|     | Kenya      | 20       | 17 (85%)   | 2.65 (±1.04) | 5.10 (±4.20)        | 0.00 | J      | 0.51 (±0.06) | 0.5 (±0.05)  | -0.025          | P<0.01   |
|     | Tanzania1  | 20       | 20 (100%)  | 4.65 (±1.57) | 7.50 (±4.66)        | 0.00 | J      | 0.64 (±0.04) | 0.65 (±0.03) | 0.019           |          |
|     | Tanzania2  | 20       | 20 (100%)  | 3.65 (±1.18) | 5.65 (±3.69)        | 0.00 | J      | 0.56 (±0.05) | 0.57 (±0.04) | 0.018           |          |
|     | Zambia     | 20       | 20 (100%)  | 4.75 (±1.74) | 6.30 (±4.11)        | 0.15 | J+K    | 0.57 (±0.05) | 0.69 (±0.03) | 0.182 (P<0.01)  |          |
|     | Namibia    | 20       | 20 (100%)  | 3.45 (±1.00) | 6.40 (±4.32)        | 0.05 | L      | 0.56 (±0.04) | 0.57 (±0.03) | 0.011           |          |
|     | RSA1       | 20       | 20 (100%)  | 4.00 (±1.08) | 5.95 (±3.61)        | 0.05 | J      | 0.61 (±0.06) | 0.66 (±0.03) | 0.082 (P<0.05)  | P<0.01   |
|     | RSA2       | 20       | 20 (100%)  | 4.60 (±1.27) | 6.65 (±4.39)        | 0.25 | J+M    | 0.69 (±0.04) | 0.69 (±0.03) | -0.002          |          |
|     | India      | 20       | 5 (25%)    | 1.45 (±0.89) | 0.95 (±0.84)        | 0.10 | N      | 0.11 (±0.05) | 0.13 (±0.05) | 0.095           |          |
| 2   | Ethiopia1  | 10*      | 8 (80%)    | 3.00 (±1.56) | 3.00 (±2.48)        | 0.05 | H      | 0.45 (±0.11) | 0.49 (±0.09) | 0.165 (P<0.05)  | P<0.01   |
| 3   | Senegal    | 12**     | 12 (100%)  | 3.33 (±1.56) | 5.42 (±3.32)        | -    | A      | 0.63 (±0.08) | 0.54 (±0.07) | -0.079          |          |

**Supplemental Table S9.** Nei’s (corrected) genetic distances for microsatellite (below diagonal) and mtDNA data (above diagonal) of 14 lion populations, and results of Mantel tests, including all populations, and excluding India.

|           | Benin | Cameroon1 | Cameroon2 | Chad  | DRC   | Ethiopia2 | Kenya  | Tanzania1 | Tanzania2 | Zambia | Namibia | RSA1   | RSA2   | India  |
|-----------|-------|-----------|-----------|-------|-------|-----------|--------|-----------|-----------|--------|---------|--------|--------|--------|
| Benin     | -     | 4.435     | 3.345     | 3.511 | 3.412 | 13.145    | 10.075 | 10.075    | 10.075    | 10.670 | 13.136  | 10.075 | 14.166 | 4.014  |
| Cameroon1 | 0.789 | -         | 0.418     | 1.922 | 1.822 | 15.615    | 12.531 | 12.531    | 12.531    | 13.129 | 15.604  | 12.531 | 16.643 | 6.451  |
| Cameroon2 | 0.801 | 0.491     | -         | 0.334 | 0.735 | 14.516    | 11.435 | 11.435    | 11.435    | 12.032 | 14.505  | 11.435 | 15.540 | 5.360  |
| Chad      | 0.716 | 0.619     | 0.600     | -     | 0.902 | 14.679    | 11.599 | 11.599    | 11.599    | 12.197 | 14.669  | 11.599 | 15.700 | 5.525  |
| DRC       | 0.902 | 0.493     | 0.571     | 0.718 | -     | 14.575    | 11.497 | 11.497    | 11.497    | 12.094 | 14.565  | 11.497 | 15.600 | 5.425  |
| Ethiopia2 | 1.900 | 1.794     | 1.311     | 1.089 | 1.344 | -         | 9.077  | 9.077     | 9.077     | 9.672  | 12.137  | 9.077  | 13.170 | 15.189 |
| Kenya     | 1.091 | 0.957     | 1.362     | 1.085 | 1.175 | 1.225     | -      | 0.000     | 0.000     | 0.584  | 9.071   | 0.000  | 10.099 | 12.107 |
| Tanzania1 | 0.872 | 0.805     | 0.927     | 1.042 | 0.783 | 1.353     | 0.373  | -         | 0.000     | 0.584  | 9.071   | 0.000  | 10.099 | 12.107 |
| Tanzania2 | 0.659 | 0.903     | 1.014     | 0.963 | 0.820 | 2.018     | 0.463  | 0.196     | -         | 0.584  | 9.071   | 0.000  | 10.099 | 12.107 |
| Zambia    | 0.630 | 0.772     | 0.839     | 0.959 | 0.629 | 1.539     | 0.729  | 0.358     | 0.518     | -      | 9.665   | 0.000  | 10.694 | 12.705 |
| Namibia   | 0.920 | 1.129     | 1.022     | 1.081 | 0.989 | 1.488     | 1.065  | 0.834     | 0.932     | 0.631  | -       | 9.071  | 5.028  | 15.179 |
| RSA1      | 0.997 | 0.991     | 0.804     | 0.998 | 0.874 | 1.217     | 0.882  | 0.493     | 0.768     | 0.523  | 0.506   | -      | 10.099 | 12.107 |
| RSA2      | 1.289 | 1.027     | 1.050     | 1.261 | 1.115 | 1.458     | 0.837  | 0.612     | 0.888     | 0.601  | 0.511   | 0.467  | -      | 16.201 |
| India     | 1.990 | 1.493     | 1.918     | 1.789 | 1.228 | 2.193     | 1.065  | 1.589     | 2.098     | 1.724  | 1.922   | 1.683  | 1.775  | -      |



**Supplemental Information S1.** Details on sample storage, DNA extraction, PCR, fragment analysis and sequencing.

### Permits

No samples were collected specifically for this study, and all included samples had been collected during previous fieldwork and stored in biobanks. More detailed information about permits and issuing authorities for each included population is given below:

Benin: Direction Generale des Forets et Ressources Naturelles – DGFRN (National Directorate for Forests and Natural Resources), local park authorities Pendjari Biosphere Reserve: samples were collected during previous fitting of GPS collars on lions (publication in prep. by Sogbohossou *et al.*), no individuals were handled for this study.

Cameroon: Ministry of Environment and Forestry, local park authorities Waza National Park and Bénoué Ecosystem: samples were collected during previous fitting of GPS collars on lions (publication by Tumenta *et al.* (2009)), no individuals were handled for this study.

Chad: Ministry of Environment, Water, & Fisheries, local park authorities Zakouma National Park: samples were collected during previous studies, no individuals were handled for this study.

DRC: Institut Congolais pour la Conservation de la Nature – African Parks Network, Garamba National Park management: samples were collected during previous fitting of GPS collars on lions, no individuals were handled for this study.

Zambia: Zambia Wildlife Authority (ZAWA): samples were collected during previous studies (publication by Dubach *et al.* (2013)), no individuals were handled for this study.

RSA: South African National Parks (SANParks): samples were collected during previous studies, no individuals were handled for this study.

Kenya: Kenya Wildlife Service (KWS): samples were collected during previous fitting of GPS collars on lions (publication in prep. by Jirno *et al.*), no individuals were handled for this study.

Ethiopia (captive), Tanzania, Namibia, RSA: samples were obtained from the IZW Leibniz Institute for Zoo and Wildlife Research (Germany). Samples were collected during previous studies (publications by Driscoll *et al.* (2002) and Bruche *et al.* (2012)), no individuals were handled for this study.

India: samples were obtained from National Cancer Institute (NCI) (U.S.A.): samples were collected during previous studies (publication by Driscoll *et al.*, 2002), no individuals were handled for this study.

### Sample storage and DNA extraction

Blood and tissue samples were preserved dried (Zambia) or in buffer (0.15 M NaCl, 0.05 M Tris-HCl, 0.001 M EDTA, pH = 7.5) and stored at -20°C. DNA was extracted using the Dneasy Blood & Tissue kit (Qiagen) following the manufacturer's protocol.

### Microsatellite analysis

Twenty microsatellite loci, originally developed for domestic cat (Menotti-Raymond *et al.* 1999), were selected from a set that had previously been used in lion (Driscoll *et al.* 2002) (see Supplemental Table S2). A selection was made based on high variability, high amplification success and no apparent presence of null alleles. To enable resizing to already published datasets from Driscoll *et al.* (2002) and Bruche *et al.* (2012), four samples of these studies (Tanzania10 and RSA10; Ethiopia12 and Ethiopia13) were included for all 20 microsatellites. During PCR the products were fluorescently labelled (HEX, TAMRA and FAM) by adding M13 tails to the 5' end (Schuelke 2000). PCR reactions contained 0.75 mM MgCl<sub>2</sub>, 0.4 mg/ml bovine serum albumin, 10x PCR buffer, 200 µM dNTPs, 0.1 U/µl Taq polymerase, 0.4 µM of both amplification primers and the M13 fluorescently labeled primer, and 1 µl of DNA template in a total volume of 15 µl. The PCR reaction was performed using an initial denaturation step of 94°C for 4min, followed by 40 cycles of 94°C for 20s, 51°C for 1 min, 72°C for 1 min and a final extension step of 72°C for 10 min. PCR products with different labels and non-overlapping fragment sizes (min. 20 bp difference between longest and shortest allele documented) were pooled and

run on a MegaBACE sequencer (GE Health Care, Eindhoven, The Netherlands) or ABI3730XL (Macrogen Inc., Amsterdam, The Netherlands) with ET-ROX400 or ROX400 as an internal standard. To enable comparisons between runs and machines, we included a minimum of two known samples for every locus in each run. Allele lengths were scored using MegaBace Fragment Profiler version 1.2 (Amersham Biosciences, 2003) or Peak Scanner Software v1.0 (Life Technologies). Samples with a weak or distorted signal were re-amplified and were included in a subsequent run.

Microsatellite data were checked for potential null alleles and allelic dropout using Microchecker (Van Oosterhout *et al.* 2004). The data were tested for linkage disequilibrium using the Fisher's exact test in GENEPOP 4.2.1 (Raymond & Rousset 1995), applying 10000 dememorisations, 100 batches and 5000 iterations per batch as Markov chain parameters.

### mtDNA analysis

PCRs were performed with three primer pairs (Supplemental Table S2), designed with the web-based software Primer3v 0.4.0 (Rozen & Skaletsky 2000). PCR reactions contained 1 mM MgCl<sub>2</sub>, 0.4 mg/ml bovine serum albumin, 10x PCR buffer, 200 µM dNTPs, 0.1 U/µl Taq polymerase, 0.4 µM of both amplification primers and 1 µl of DNA template in a total volume of 20 µl. The PCR program consisted of an initial denaturation step of 94°C for 4min, followed by 40 cycles of 94°C for 20s, annealing temperature ranging from 51°C to 54°C depending on the primer set, for 1 min, 72°C for 1 min and a final extension step of 72°C for 10 min. Sequencing was performed by Macrogen Inc., Amsterdam, The Netherlands.

### Quality control

Out of a total of 2188 data points (Dataset 1: 104 samples \* 20 loci + 4 samples (Chad) \* 15 loci + 4 samples (Ethiopia2)\* 12 loci), Dataset 1 included a total of 28 missing genotypes (1.28%). None of the individuals had missing values at more than two loci. Indications for stuttering errors or null-alleles, as is suggested by the general excess of homozygotes in Microchecker, were identified in three populations for one locus (FCA178 in Ethiopia1 and Namibia; FCA211 in RSA1) and in the Zambia population for six loci (FCA026, FCA057, FCA094, FCA208, FCA211 and FCA224). However, in the case of the Zambia population this is likely to be the result of genetic structure within the population (see results STRUCTURE analysis). Since none of the loci were consistently positive for more than two populations, we included all loci in downstream analyses. There was no indication of allelic dropout. Pairwise comparison of loci in each population identified significant linkage (P<0.05) in 37 cases in a total of 2850 comparisons (1.30%). No loci were consistently in linkage disequilibrium across populations and a pairwise comparison between loci on the entire dataset did not reveal significant linkage.

### References

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Supplemental Information S2. Details on DNA extraction, PCR, fragment analysis for Dataset 3.

Permits

Senegal: No specific permits were required for the collection and export of samples from Senegal. The samples were collected as part of a lion survey carried out jointly with the Senegalese national park authority (Direction des Parcs Nationaux; DPN). DPN waived all permit requirements, to facilitate the timely assessment of lion population status in the park, including of its genetic makeup. Samples were collected non-invasively (scat), no individuals were handled for this study.

DNA extraction & species identification

Genomic DNA was extracted from scat samples using the QIAmp DNA Stool Mini Kit (QIAGEN, Valencia, California, USA) following the manufacturer’s protocol for isolation from stool for human DNA analysis with some modifications to improve DNA quality and quantity. All instruments were cleaned with DNAaway™ (Molecular BioProducts, San Diego, California, USA) and placed in an ultraviolet cross-linker prior to use. Additionally, all extractions were performed on a dedicated lab bench and in a pre-PCR laboratory to avoid contamination. Two microcentrifuge tubes were prepared with approximately 180 – 200 mg per sample in each using shavings from the outer layer of the scat sample. Samples were incubated overnight (approximately 18 h) at 56 °C on a rotator (approximately 22 RPM) with 1.5 mL of ASL buffer. All of the supernatant for each unique sample was combined into two tubes, with one tube containing 1.5 mL of supernatant and the other tube having all remaining supernatant up to 1.5 mL. A proportionate amount of InhibitEx tablet was added to the second tube of supernatant if the volume was less than 1.5 mL. Cold ethanol was used and AE elution buffer was heated to 70°C prior to addition to the spin-column membrane. Elution of DNA from the spin-column membrane was carried out in three stages, with the addition of 60 µl of AE buffer each time and a 40 minute and two 15 minute incubations at room temperature prior to centrifugation, for a total elution volume of 180 µl.

All scat samples were screened for species identification using species-specific primers amplifying regions of four mitochondrial genes as described in Caragiulo *et al.* (2013) (Table S2-1). DNA amplifications from all extractions were carried out in 25 µl reaction volumes containing 22.3 µl of ultrapure water, 0.7 µl of forward primer (10 µM), 0.7 µl of reverse primer (10 µM), 0.3 µl bovine serum albumin (BSA), one illustra™ puReTaq Ready-To-Go PCR Bead (GE Healthcare, Piscataway, New Jersey, USA), and 1.0 µl of template DNA.

**Table S2-1.** Four mitochondrial gene regions spanning 1,140 bp were amplified using the primer sets described below. The Carnivorous primers amplify a region nested within the region amplified by the Canideos primers.

| Type  | Location     | Primername              | Annealing Temp (°C)         | Origin                     |
|-------|--------------|-------------------------|-----------------------------|----------------------------|
| mtDNA | Cytochrome b | Carnivorous F: H15149   | 50                          | Kocher <i>et al.</i> 1989  |
|       |              | Carnivorous R: Farrel-R |                             | Farrell <i>et al.</i> 2000 |
|       |              | Canideous F: H15149     | 52                          | Kocher <i>et al.</i> 1989  |
|       |              | Canideos R: Canid-L1    |                             | Paxinos <i>et al.</i> 1997 |
|       | 12S rRNA     | L1085                   | 57                          | Kitano <i>et al.</i> 2007  |
|       |              | H1259                   |                             | Kitano <i>et al.</i> 2007  |
|       | 16S rRNA     | 16Scp-F                 | 52.5-51.5 (touchdown cycle) | Pomilla <i>et al.</i> 2009 |
|       |              | 16Scp-R                 |                             | Pomilla <i>et al.</i> 2009 |
|       |              | L2513                   | 57                          | Kitano <i>et al.</i> 2007  |
|       |              | H2714                   |                             | Kitano <i>et al.</i> 2007  |
|       | ATPase-6     | ATP6-DF3                | 50                          | Chaves <i>et al.</i> 2012  |
|       |              | ATP6-DR2                |                             | Chaves <i>et al.</i> 2012  |

All samples were visualized on a 2.0% agarose gel in TBE buffer and PCR products were purified using the Agencourt AMPure XP PCR purification protocol (Beckman Coulter, Indianapolis, Indiana, USA). Purified PCR products were then sequenced using the Big Dyeterminator protocol (Applied Biosystems, Carlsbad, California, USA). Sequencing reactions were carried out in 8.0 µl reaction volumes containing 4.75 µl ultrapure water, 0.75 µl extension buffer, 0.5 µl Big Dye, 1.0 µl primer (1.6 µM), and 1.0 µl of purified PCR product. The sequencing reaction was carried out separately for the forward and reverse primers. The thermocycler profile for all sequencing reactions followed that of Platt *et al.* (2007). Sequencing amplifications were purified using the Agencourt CleanSEQ Dye Terminator Removal protocol (Beckman Coulter, Indianapolis, Indiana, USA) and sequenced in an ABI 3730xl DNA Analyzer (Applied Biosystems, Carlsbad, California, USA). Sequences were manually edited using Sequencher (version 5.0, Gene Codes Corporation, Ann Arbor, Michigan, USA) and Geneious (Drummond *et al.*, 2012), and compared to both an in-house database of carnivore mtDNA sequences and the NCBI nucleotide BLAST database to confirm species identification. All samples identified as lion were used in further analyses.

The 12 microsatellite loci used in this study (FCA032, FCA075, FCA096, FCA100,FCA124, FCA126, FCA132, FCA208, FCA212, FCA225, FCA229, FCA275) were adapted from a genetic map of the domestic cat (Menotti-Raymond *et al.*, 1999) and optimized for lions. PCR reactions were carried out in 20.0 µl multiplex reactions containing 5 µl of extracted DNA, 0.20 – 1.60 µl of each forward and reverse 10 µM primer, 10.0 µl Qiagen Mutlplex PCR Master Mix, 2.0 µl Q-solution, and the remaining volume was RNase-Free water (Qiagen, Valencia, California, USA). Primers were grouped into five multiplex reactions based upon fluorescent tag and amplicon size (Table S2-2). Thermocycling conditions were the same for each multiplex, except for the touchdown and annealing temperature, and were as follows: 95 °C for 15 minutes, 13 cycles of 94 °C for 30 seconds, touchdown annealing temperature for 1.5 minutes, and 72 °C for 1 minute, followed by 32 cycles of 94 °C for 30 seconds, annealing temperature for 1.5 minutes, and 72 °C for 1 minute, followed by 30 minutes at the annealing temperature (Table S2-2). Samples were prepared for analysis by mixing 1 µl of PCR product with 9 µl of an 8.82 µl: 0.18 µl mixture of Hi-Di formamide: GeneScan 500 LIZ size standard (Applied Biosystems, Carlsbad, California, USA). Samples were heat-shocked for 3 minutes at 95 °C and genotypes were analyzed using an ABI 3730xl DNA analyzer (Applied Biosystems, Carlsbad, California, USA). Genotypes were scored with GeneMapper v. 4.0 software (Applied Biosystems, Carlsbad, California, USA) and individually verified by visual inspection.

**Table S2-2.** FCA primers were grouped into five multiplexes and thermocycling conditions were optimized for each multiplex.

| Group       | Included loci | Touchdown Cycle<br>Annealing Temperature (°C) | Annealing Temp (°C) |
|-------------|---------------|-----------------------------------------------|---------------------|
| Multiplex 1 | FCA032        | 60.4 - 0.3                                    | 58                  |
|             | FCA100        |                                               |                     |
|             | FCA124        |                                               |                     |
| Multiplex 2 | FCA126        | 62.4 - 0.3                                    | 60                  |
|             | FCA212        |                                               |                     |
|             | FCA229        |                                               |                     |
| Multiplex3  | FCA096        | 59.4 - 0.3                                    | 57                  |
|             | FCA132        |                                               |                     |
|             | FCA275        |                                               |                     |
| Multiplex 4 | FCA075        | 59.4 - 0.3                                    | 57                  |
|             | FCA208        |                                               |                     |
| Multiplex 5 | FCA225        | 57.4 - 0.3                                    | 55                  |

All microsatellite amplifications were performed at least four times using the multi-tube approach (Taberlet *et al.*, 1996) to identify possible allelic dropout. Allelic dropout and PCR success was quantified per locus using GIMLET (Valière, 2002). Consensus genotypes were defined for each sample by comparing results from both a consensus genotype inference method using GIMLET (Valière, 2002) and manual inspection. All samples that did not produce reliable consensus genotypes for at least 6 loci were excluded from further analyses.

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