A CENTURY OF CONSERVATION GENETICS OF THE LION (*Panthera leo*)

A Dissertation

by

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ABSTRACT

Lions are a flagship species, meaning that as a large, charismatic carnivore, research and conservation for the lion influences many other species that share its habitat. Proper management requires understanding of a species' genetic history and the amount of variation that exists across its range. Knowing trends in genetic diversity in the lion will help in making decisions on where and how to manage lions and other wildlife populations. With the use of modern biotechnology, I determined the genetic architecture of both historical (>100 years ago) and modern (2000 to present) lion populations across the traditional range states in Africa and Asia. Both datasets were analyzed using the same methods allowing for a more direct comparison over time than has previously been employed. DNA was isolated from high quality and well-documented museum specimens as well as biological material collected from contemporary lions and data from several recently published studies. Fourteen microsatellite molecular markers were redesigned to be specific to the lion then paired with mitochondrial DNA analyses to determine genetic diversity. Genetic diversity was examined to assess local structure in Zambia and to determine range-wide changes to the overall population over the past century. The historical population of lions was panmictic. But, while most of the current subpopulations of lion assessed in this study are considered to be genetically healthy, they exhibit fine population structure. The dramatic differences between the population structure of the historical and modern population illustrates our need to better understand relationships between the now fragmented subpopulations. Knowledge about the genetic health of the lion has helped us to identify existing wild lion subpopulations that are most at risk and can now be used to make recommendations to guide management actions to safeguard the future genetic health of the lion.

DEDICATION

To Paughey

#gooddog

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Contributors

This work was supervised by a dissertation committee consisting of Professors James Derr of the Department of Veterinary Pathobiology [advisor], William Murphy of the Department of Veterinary Integrative Biosciences, and Bruce Budowle of the University of North Texas Health Science Center, and Professors Emeritus Gus Cothran of the Department of Veterinary Integrative Biosciences and James Woolley of the Department of Entomology.

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CHAPTER I

INTRODUCTION

The African lion (*Panthera leo*) is one of the most iconic and recognizable species in the world[1] (Figure 1.1). It is used worldwide to represent bravery, strength and power and exemplifies an entire continent as a member of Africa's "Big 5". Although this animal symbolizes authority, the lion has been pushed out of its historic home range as a result of anthropogenic changes to the African landscape. The major sources of lion mortality across its range have been identified as being human encroachment into lion habitat and resultant habitat destruction and land conversion, reduction of prey base, and retaliatory (or pre-emptive) killing in response to conflict[2,3] as well as a growing industry in the trade of lion products[4]. The human population in Africa has nearly quadrupled over the last 50 years (Figure 1.2) and is expected to again double by 2050[5]. Rapid human population growth expanding across Africa and the fact that lion numbers have been shown to be inversely related to human population density, conservation concerns are justified. But this trend is primarily speculation based on imprecise comparisons to historic records so the actual impact to the lion population is unknown.

Figure 1.1. Male and female lion. Photo from Adobe Spark.

Demographic information available on the African lion is often inconsistent which can compromise conservation decisions. Scientific estimates of historic population sizes vary widely, with some ranging from 30,000 to 100,000 individuals[6] estimated using very different techniques during different time periods. Estimates reported in the media vary even more ranging from as low as 16,500 to as high as 200,000 individuals (Table 1.1 and Figure 1.2). Many conservation decisions are based on population declines but population declines are based off guesstimates[7] compared to a historical number that comes from an article from the 1970's[8]. These comparisons are then used to make predictions on future declines and make inferences on the survival of the species in the wild. Despite the frequency with which they are presented and their widespread

applications when drawing conclusions on population declines, there have been no attempts to validate the accuracy of historic lion population size estimates.

Table 1.1. Population estimates reported via the media and scientific publications or reports.

Type	Publication	Year	Estimate
Media	Myers 1975	1950	400,000
	National Geographic	1960	100,000
	Myers 1975	1975	200,000
	National Geographic	2012	32,000
	Safari Club International	2017	18,000-33,000
	Defenders of Wildlife	"Current"	21,000
	Lion Recovery Fund	"Current"	20,000
Scientific	Ferreras and Cousins 1996	1980	75,800
	Nowell and Jackson 1996	1996	30,000-100,000
	Chardonnet 2002	2002	39,000 (29,000-47,000)
	Bauer and van der Merwe 2004	2004	23,000 (16,500-30,000)
	IUCN 2006	2006	33,000
	Riggio *et al* 2012	2012	32,000-35,000
	Bauer *et al* 2015	2015	23,000-39,000

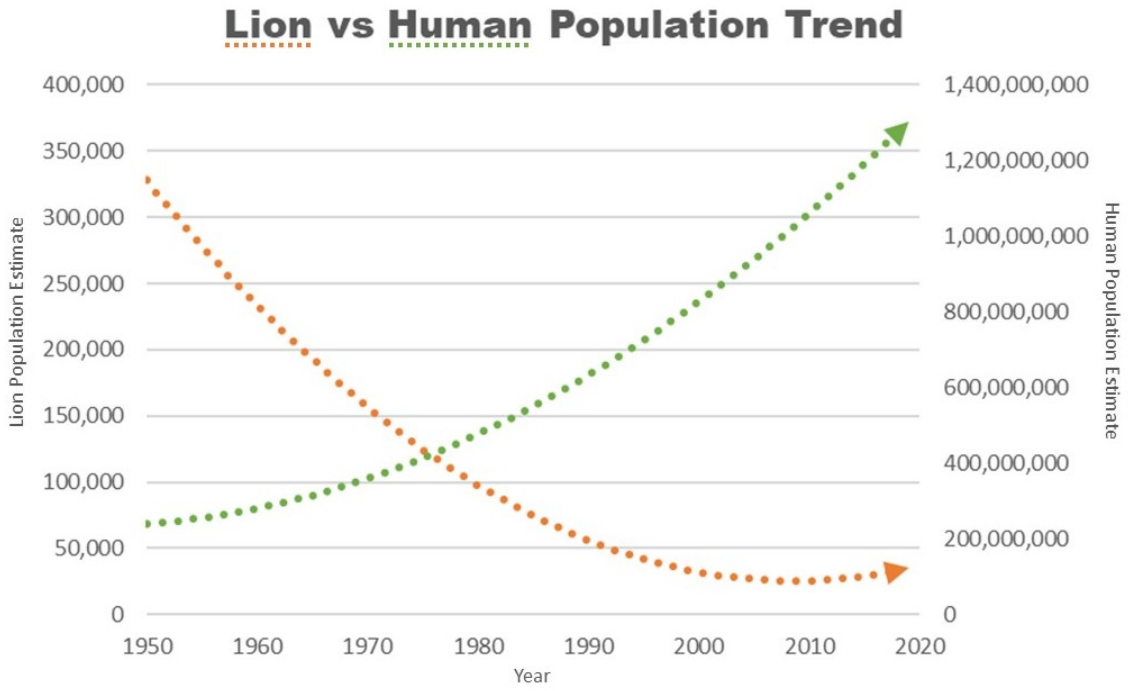

Figure 1.2. Lion versus human population trend. Human population estimates are from World Population Review (worldpopulationreview.com/continents/africa-population). For lion population estimates see Table 1.1.

This study aims to improve upon this information to aid policy makers by providing a reliable assessment of population status based on the genetic health of the overall population. Using population genetics, this study makes a direct comparison between lion populations from 100 years ago with populations of today to get an idea of how growing human populations are affecting lion populations.

DEMOGRAPHICS

The lion is a versatile and resilient species. Found in 28 countries, lions are capable of occupying a wide range of habitat types from forests to grasslands to deserts. The lion is a social carnivore and the only big cat to exist in groups, called prides[3]. Lion prides, on average, consist

of 4-6 adults[3] with 2–18 related females born to that pride and 1–7 males who migrate into the pride[9]. Female lions reach reproductive maturity at approximately 3.5 years and are reproductively active for approximately 10 years[10]. Lions have what is considered a long generation time of approximately 7 years[3,11] but are prolific breeders[12] and, under favorable conditions, can rapidly recover from population declines[13,14].

Historically, lions were found in every country in Africa except Equatorial Guinea. The once continuous range has become fragmented causing regional populations to become more isolated. Recent studies have been focusing on Lion Conservation Units or metapopulations to construct conservation programs focusing on populations within specific locations[15,16]. With translocation becoming a well-practiced technique to prevent inbreeding within populations closed to dispersal or immigration[17], it must be determined whether there needs to be a focus on maintaining genetic diversity throughout the entire population or if there needs to be a more narrowed focus to prevent the loss of regional diversity.

CONSERVATION STATUS

Most recently assessed in 2016, *Panthera leo* has a Global Red List status of Vulnerable A2abcd and a Regional Red List status of Least Concern [18]. The least concern designation is considered to be conservation dependent as populations in lion strongholds[19] heavily influence the overall assessment. Populations outside the lion strongholds qualify as Near Threatened if assessed alone.

Concerns regarding the conservation status of the lion have recently attracted global attention with publications declaring that the African lion has undergone a precipitous species population decline across its range [19–21]. From this, various national and international groups,

including some African countries, are actively supporting an uplisting of the African lion to a Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES) Appendix I status.

In July of 2015, Cecil, a regionally famous radio-collared lion from Zimbabwe's Hwange National Park, was shot under suspicious circumstances by an American trophy hunter. The incident quickly received global media coverage generating international interest around the African lion. For a few months in 2015, the public's outcries for the future of the lion were leading media stories. A petition for the United States Fish and Wildlife Service (USFWS) to list the African lion under the Endangered Species Act (ESA) had been in circulation since 2011[22] and the upswing in media coverage on the species brought about more petitions to bring a decision to action. On October 27, 2014, the USFWS formally proposed listing the lion as threatened under the ESA[23], a similar listing to the current International Union of Conservation of Nature (IUCN) listing of "vulnerable". Then on January 22, 2016, the USFWS made the official ruling to list the African lion as two subspecies under the ESA, *Panthera leo leo* and *Panthera leo melanchaita*, as endangered and threatened, respectively[24] (Table 1.2).

Table 1.2. Taxonomic recognition and status listing of governing agencies.

Organization	Scientific Name	Description	Status
CITES	Panthera leo	African Subpopulations	Appendix II
	Panthera leo persica	Asian Subpopulations	Appendix I
IUCN	Panthera leo leo	African Subpopulations	Vulnerable
	Panthera leo persica	Asian Subpopulations	Endangered
USFWS ESA	Panthera leo leo	West Africa, Central Africa, and India	Endangered
	Panthera leo melanchaita	Eastern and Southern Africa	Threatened

As mentioned above, lion populations are not affected the same across the continent. A status uplisting may not be beneficial for lion populations in all areas. In areas with thriving lion populations, an uplisting of the African lion could have deleterious effects on eco-tourism in regards to income from professional hunting excursions, game ranches which own lion, as well as future funding on lion research. Determination of genetically distinct sub-species could be used for conservation management, establishing individualized programs for each sub-population.

TAXONOMY

Lions are a member of the *Panthera* lineage in the *Felidae* family. There is much debate between scientists as to whether there are sub-species of the African lion. Over the years, scientists have given more than 30 different scientific names to the lion[25] (Table 1.3); however, although it does recognize eleven subspecies of *Panthera leo* as taxa in the Catalog of Life, the IUCN Red List has assessed only *Panthera leo* and *Panthera leo* ssp. *persica* (Asiatic Lion) to be classified under their own status[3]. This distinction was made after Gaur *et al*.[26] determined the Asiatic lion, located in India, to be a distinct sub-species through microsatellite analysis and Bagarthia *et al*[27] sequencing the whole mitochondrial genome. A phylogenetic analysis was done comparing an Asiatic lion mitogenome and a partial mitogenome of Panthera leo with that of the rest of the Panthera lineage. The results were not identical; therefore, their conclusion was that they are different sub-species.

Table 1.3. Scientific names recognized throughout time of subspecies of lion. Subspecies names may be preceded by *Felis leo*, *Panthera leo*, and/or *Leo leo*. Names in bold are subspecies currently recognized by a governing organization (See Table 1.2). Subspecies are regionally categorized by the subspecies currently recognized by USWFS ESA. Names with an asterisk (*) are considered an extinct subspecies. Subspecies within the same region are often considered synonyms of each other[28,29].

Scientific Name	*Locality*	*Region*	*Citation*
leo	**Africa**	**not specified**	**Allen, 1924**
africana	Africa	not specified	Brehm, 1829
nobilis	not specified	not specified	Gray, 1867
capensis	not specified	not specified	Fischer, 1830
leo	**Northern Africa and Asia**		**USFWS, 2016**
persica	**India**	**Asia**	**von Meyer, 1826**
asiaticus	Asia	Asia	Brehm, 1829
bengalensis	Bengal	Asia	Bennett, 1829
goojratensis	Guzerat	Asia	Smee, 1833
indica	India	Asia	de Blainville, 1843
barbarica	North Africa	North	Myer, 1826
*leo**	Barbary Coast	North	Linneaus, 1758
nigra	Algeria	North	Locke, 1867
senegalensis	Senegal	West	von Meyer, 1826
gambiana	Gambia	West	Gray, 1843
nubica	Egypt to Central Sudan	Central	de Blainville, 1843
kamptzi	Cameroon	Central	Matschie, 1900
azandica	Northern Congo	Central	Allen, 1924
bleyenberghi	Belgian Congo	Central	Lonnberg, 1914
hollisteri	Congo	Central	Allen, 1924
melanochaita	**Southern and East Africa**		**USFWS, 2016**
somaliensis	Somalia	East	Noach, 1891
massaica	Kenya	East	Neumann, 1900
nyanzae	Uganda	East	Heller, 1913
somaliensis	Somalia	East	Noach, 1891
sabakiensis	Mount Kilimanjaro	East	Lonnberg, 1910
roosevelti	Ethiopia	East	Heller, 1913
webbensies	Somalia	East	Zukowsky, 1964
krugeri	Transvaal	South	Roberts, 1929
*melanochaita**	Cape	South	Smith, 1842
verneyi	Kalahari	South	Roberts, 1948

Currently, the taxonomy recognized by the IUCN Species Survival Commission (SSC) Cat Specialist Group does not coincide with that recognized by the USFWS ESA (Table 1.2). They have, however, provisionally proposed to recognize the same taxonomic split[3] as phylogenetic studies including historical samples of Barbary lions[30] and West African lions[31] with Asiatic lions that have suggested they are closely related and may be sister species or sub-species to the sub-Saharan lion.

Previous sub-species distinctions have been based primarily on geographic variation in body size, color and mane size[25]; however, studies have shown that these variations are due in large part to physical factors such as nutrition, availability of water, and weather[32]. The Tsavo lions of Kenya are known for the males having virtually no mane while Kalahari lions have large manes. While both locations are typically quite arid, water is more readily available in the Kalahari than it is in Tsavo. The Cape lion, thought by some to be an extinct sub-species, could be identified by its lush mane. A genetic study on recently extinct "sub-species" concluded they were most likely part of the extant southern population[33]. Therefore, the lush manes of the Cape lion would be the result of higher rainfall and colder temperatures of the Cape as compared to other regions with lions. These morphological differences and the low genetic variation as compared to other Panthera species[25] have made the sub-species question a difficult one to answer.

LION RESEARCH AND GENETICS

A number of abundance and distribution studies of the African lion have been performed through the use of interviews[7], spoor counts[34], call-ups[35–37], and camera traps[35]. While genetic studies have been performed, they have been primarily phylogenetic in nature with little to no

focus on range-wide population structure[15,31,33,38–41] or restricted to a particular region [42–49]. This study determined levels of genetic diversity and population structure and estimated the historical population size of the African lion across the species' range (Figure 1.3).

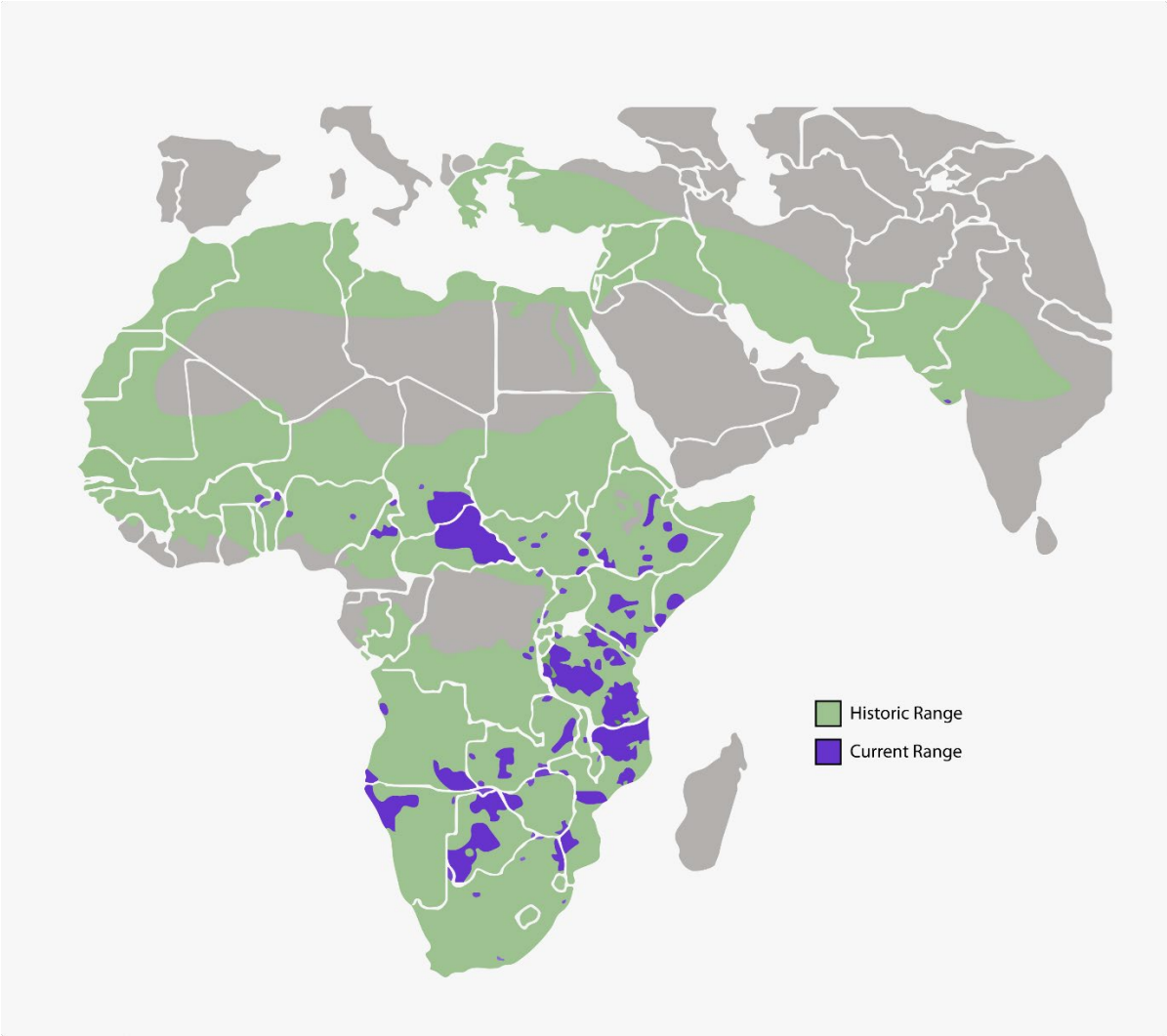

Figure 1.3. The current range of the lion overlaid on the historic range.

Advances in the conservation of lions have paralleled the advances in sequencing technology[50]. One of the first genetic projects done on lions used minisatellites to identify the

kinship within prides of lions and coalitions of lions[51]. This study was a first to link behavioral biology and genetics but used relatively rudimentary genetic techniques. When mitochondrial DNA and microsatellites became the prominent molecular markers for wildlife research, all lion studies, whether they be phylogenetic or population genetic in nature, moved to these systems. The biggest leap for lion research was the development of microsatellites for the domestic cat by Menotti-Raymond *et al*[52]. Many of the genetic studies done of big cats use these microsatellites. With next-generation sequencing becoming more cost-efficient more research is being directed toward single nucleotide polymorphisms (SNP) discovery and analysis of whole genomes. Various research groups have been working on a full genomic assembly but a finished assembly is yet to be made publicly available (private communication).

Phylogenetic studies using a variety of genes across the mitogenome show mitochondrial haplotypes cluster regionally. Genetic studies that include structural analyses based on microsatellites also show lions group into regional populations, likely a result of genetic isolation. Analysis of mtDNA gene cytochrome b[15,31,40] supports that the lion consists of five major phylogeographical groups: North African/Asian, West African, Central African, East African, and South African (Figure 1.4). In Barnett *et al*.[30], the Asiatic lion clustered with the extinct population from North Africa. North Africa clustered with West and Central Africa, agreeing with the Asiatic lion being clustered within the Central/Western Africa clade. The Eastern/Southern Africa clade consists of individuals from East Africa and eastern Southern Africa. This analysis included southern East African countries, Zambia and Zimbabwe, which clustered with eastern Southern African countries. Barnett *et al*.[30] only included northern East Africa, Somalia and Ethiopia, in their analysis which could be the reason for the distinction between East and Southern African clades.

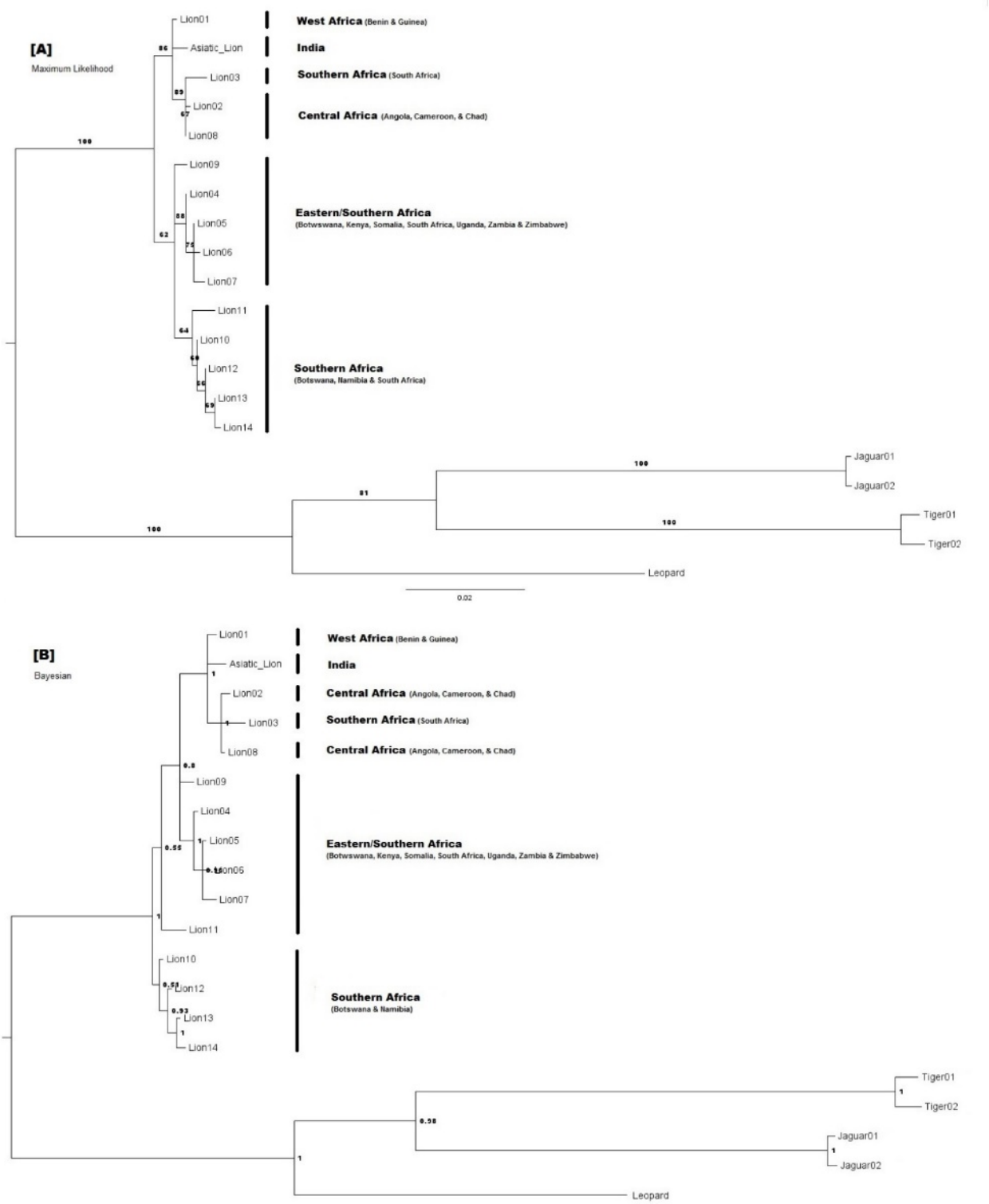

Figure 1.4. Phylogenetic analyses of cytochrome b sequences available from NCBI. [A] Maximum Likelihood analysis of using GARLI[53] with bootstrap values on the branches. [B] Bayesian analysis using MrBayes[54] with posterior probability values on the nodes.

Antunes *et al*.[38] looked at the phylogenetic relationships of the feline immunodeficiency virus across the entire range of the lion. The 6 distinct subtypes of the virus were shown to correspond well with designated geographic regions suggesting a single interbreeding population hypothesis to be incorrect. Any sub-species distinction, however, was not made due to conflicting results between the analyses of paternally inherited nuclear DNA (sex-determining region Y) versus maternally inherited mtDNA.

Several studies[30,39,55] have looked at the phylogeography of the lion across its range but over time, comparing modern lions with its extinct counterparts. The 2009 study[55] determined there to be three distinct taxa of lion: modern, extinct cave and extinct American. No focus was put on detail within each grouping. The 2014 study[30], aimed at finding the evolutionary origin of the modern lion, dated a "modern sub-species" of lion to 124,000 years ago originating in Eastern-Southern Africa and deemed all other regional sub-species as having gone extinct. Looking strictly at modern specimens from the same locations results in similarly distributed regional differences meaning the differences could be remnants of this evolutionary history.

Using genetic information from both historical and modern samples we can create a baseline for genetic health by making a direct comparison between populations from two time periods and assess how genetic health has been affected; if they have lost, gained or maintained diversity. We are able to estimate the effective population size (N_e) and compare how many individuals there were 100 years ago it to how many there are today using the same technique. This allows for a more direct comparison over time than has been previously employed. This study utilized much of the technology available for wildlife research including Sanger sequencing of mtDNA, genotyping of microsatellites, and SNP discovery through next generation sequencing.

NUMTS

Numts, copies of the mitochondrial sequence within the nuclear genome caused by horizontal transfer, are present within the *Felidae* family[56]. The domestic cat has a numt which spans approximately 8kb of its mitogenome and is present as a tandem repeat on the D2 chromosome. This occurred as a separate transfer event than a numt found in the Panthera lineage. The domestic cat numt occurred approximately 1.8 MYA while the Panthera numt is in parallel with its divergence about 3.45 MYA[56]. The Panthera numt can be found on the F2 chromosome and is not a tandem repeat. It is large, at approximately 12.5kb of the 17kb mitogenome, and spans from the mid NADH sub 5 to the ATP8 subunit gene (Figure 1.5). It covers 8 genes and multiple tRNAs but is highly divergent from the mitochondrial sequence.

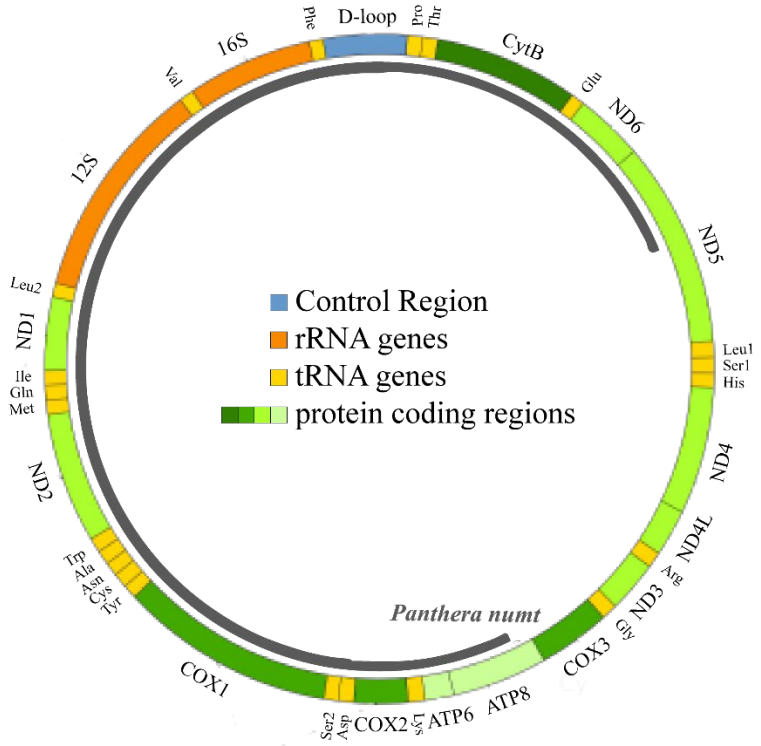

Figure 1.5. Map of the mitochondrial genome with the relative position of the Panthera numt.

Numts can be a potential source of error during analysis. Different selective constraints on mtDNA versus nDNA, such as mutation rate, cause the transferred copy to undergo different evolutionary processes resulting in different sequences. Diversity can appear to be greater than it appears if the numt is missed. False sequences of mtDNA/numt recombinants produced during PCR[57] can also result in inaccurate levels of genetic diversity. The identification and exclusion of numts greatly cleared up discordance in the Panthera phylogeny[58]. Great care was taken in this study to eliminate numts (see Chapter V).

OBJECTIVES

With the advent of polymerase chain reaction (PCR) coupled with the capability of isolating genetic material (DNA) from historic samples, genetic information can now be accessed from both long dead individuals and their contemporary counterparts. Combining these datasets will provide quantitative measures which will be used to assess the extent of change in the genetic diversity of lions over the past 100 years as well as identify existing wild lion populations that are most at risk of a decline in genetic diversity. This information will allow us to make recommendations to guide management action to safeguard future genetic health. The objectives covered in this dissertation are as follows:

1. **Estimate population size of the African lion.**

   Effective population size (N_e) is the size of the ideal population if it were going through the same amount of genetic drift as the observed population. Or to be put more simply, the effective population represents the number of individuals who will contribute genes to the next generation. Effective population size is used for captive breeding programs

and wildlife management. It is often more informative than a census population size because it provides more information as to how the population will fair in future generations.

2. **Determine how the levels of genetic diversity of lions compare over time and how they are affecting the genetic health of current populations.**

Levels of genetic diversity are directly proportional to a species' ability to adapt, survive and thrive. Therefore, loss of genetic diversity is detrimental to overall population health and long-term survival because it decreases its potential to adjust to an ever-changing environment. Genetic health relates to genetic diversity through inbreeding, genetic drift and migration of the species, all of which could affect the long-term viability of the population. For the African lion, populations isolated in national parks and private reserves may have little to no opportunity for movement across protected archipelagos[59] potentially causing an increase in inbreeding and decrease in allelic diversity. These adverse effects of low genetic diversity have been observed in small populations of African lions that exist in heavily managed fenced reserves[17]. It is difficult, however, to predict how losses in the genetic diversity within a wild lion population will negatively impact its overall health, particularly with increasingly isolated and managed populations.

3. **Document regional differences in genetic diversity in lion populations prior to the extensive management and translocation efforts of the last 100 years and determine if these differences are still present in current populations.**

Population structure is an important piece of information when forming conservation management. Knowledge of whether individuals are moving in and out of a population is integral to a successful management plan. F-statistics provides information on population structure. Each calculation provides insight on the statistical relationship individuals and populations have with one another. There are three statistics: F_{IS}, F_{ST} and F. F_{IS} is the individual within the population, F_{ST} is the differentiation between populations and F is the inbreeding coefficient. F_{ST} is a measure of genetic drift. Genetic drift works in opposition to migration. When you have a high value of F_{ST} (0.25+) this generally means there is little to no migration of individuals outside of that population. With these statistics the amount of genetic drift and migration can be determined for the population and an effect of inbreeding can be established.

Levels of genetic diversity over time will be directly compared in modern and historic samples to provide a baseline for determining the genetic health of current populations. Regional differences in genetic diversity that exist in lion populations prior to the extensive management and translocation efforts of the last 100 years will be documented to determine if these efforts are affecting African lion genetic diversity. By recreating the historical picture that represents a time when African lion populations were presumed to be robust[8], this approach provides a framework from which the genetic health of current lion populations can be assessed.

SIGNIFICANCE

This study aims to access levels of genetic differentiation and diversity across time (years) and space (geographical distance). Through analysis of mtDNA and microsatellites,

inferences about population structures and differences can be made to detect historic changes in diversity, the effects of population fragmentation, and genetic exchange between populations.

By reconstructing a genetic picture of historic lion populations and comparing these to modern day lions, a framework will be established from which the extent and effect of future changes in lion population genetics may be predicted. These results will inform management decisions that will in turn be used to safeguard the future health of wild lion populations. The information that will be obtained in this study is critical for establishing science-based management policies for long-term lion conservation.

CHAPTER II

DEVELOPMENT OF LION MINISTRS FOR USE WITH MODERN AND HISTORICAL DNA SAMPLES*

INTRODUCTION

Microsatellites, also known as short tandem repeats (STRs), are a commonly used genetic marker in wildlife research[60,61] and wildlife forensic casework[62–64]. Conservation of the flanking sequence across closely related species[65] makes cross-species amplification possible when microsatellites for a target species are unknown. However, studies have found that with increased genetic distance there is a precipitous decline in the ability to amplify microsatellite markers developed for related species[66–70].

For the lion (*Panthera leo*), genetic research has relied on genomic information originally derived from the domestic cat (*Felis catus*)[52,71] and domestic cat microsatellites are currently the primary marker used for nuclear analysis in lions. Since 2002, twelve genetic studies of the lion have used microsatellites[15,38,41,42,44,46,72–76]. The lion and the domestic cat diverged 10.8 million years ago[77] potentially introducing enough differences that some loci are problematic when not optimized. Non-specific matching from genomic differences between the lion and domestic cat can result in inconsistent amplification, null alleles, and allelic drop out creating issues for downstream analysis[78]. Differences between species can lead to changes in annealing temperature during polymerase chain reaction (PCR)[79] and similar annealing temperatures are

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* Reprinted with permission from "Development of lion miniSTRs for use with modern and historical DNA samples" by Curry, C.J. & Derr, J.N. African Journal of Wildlife Research, 49:64–74 (2019). doi:10.3957/056.049.0064

needed across loci for accurate amplification in multiplex PCR[80]. There is also no consensus for which of the 246 autosomal domestic cat microsatellites[52] should be used for lion research. In total, 107 different microsatellite loci have been used across the twelve studies (Appendix C1), making data sharing and comparison of results difficult.

Wildlife research often uses sampling methods that can result in damaged or degraded DNA[81–83], such as from non-invasive sampling, forensic samples, or historical samples from museums. This can further compound issues when using cross-species markers that were developed for larger fragment sizes. Miller *et al.*[74] evaluated a set of 28 domestic cat microsatellites for lion genotyping but they did not make comparisons back to the species from which the primers originated. The study recommends loci for use in forensics and population studies, however, many of the loci are larger in size (150+ bp) and larger loci could be problematic when using damaged or degraded DNA.

Microsatellites redesigned to amplify shorter sequences closer to the target repeat, called miniSTRs, are used in forensics as a way to create DNA profiles when working with potentially degraded samples[84–89]. To increase specificity and reliability for lion genetic research, particularly in the use of samples that may contain degraded DNA, primers of 17 previously used microsatellites were redesigned to be closer to the target repeat using lion genomic sequences. By redesigning previously used microsatellites, we maintain the ability to utilize data from previous studies.

Table 2.1. List of candidate loci results from testing miniSTRs (N=30) with observed heterozygosity (H_{OBS}) and highest reported number of alleles (A). If more than one H_{OBS} was reported from previous studies, ≤ the highest of the reported values is shown. Chromosome location (Chrom) is based on the domestic cat[52,90]. Studies that used the candidate loci are 1: Antunes *et al* 2008 (N=357), 2: Bertola *et al* 2015 (N=48), 3: Driscoll *et al* 2002 (N=60), 4: Dubach *et al* 2013 (N=480), 5: Lyke *et al* 2013 (N=90), 6: Miller *et al* 2014 (N=361), 7: Morandin *et al* 2014 (N=157), 8: Spong *et al* 2002 (N=70), 9: Tende *et al* 2014 (N=18). Only 9 of 12 studies are included here. Miller *et al* 2015 used a subset of the loci used in Miller *et al* 2014. Tensen *et al* 2018 and van Hooft *et al* 2018 were published after the miniSTRs were designed. No data available (--). (Reprinted with permission from Curry & Derr, 2019)

		Previous Lion Studies				Redesigned miniSTRs		
Locus	Chrom	Studies	H_{OBS}	A	Locus	Size (bp)	H_{OBS}	A
FCA006	D3	1,3,7	--	10	Leo006	80-130	0.77	11
FCA008	A1	1,3,6,7,8,9	≤0.72	6	Leo008	111-133	0.83	8
FCA026	D3	2,3,4,5,6,8,9	≤0.84	9	--	--	--	--
FCA031	E3	6,7,8,9,10	≤0.7	9	Leo031	186-200	0.47	7
FCA045	D4	3,4,5,7,8,9	≤0.67	5	Leo045	80-110	0.27	6
FCA077	C2	1,3,4,5,7,8,9	≤0.77	7	Leo077	98-112	0.90	8
FCA085	E2	1,2,3,6,7	0.60	6	Leo085	72-94	0.63	7
FCA091	B4	1,2,3,7	--	11	--	--	--	--
FCA096	E2	3,4,5,6	≤0.76	5	--	--	--	--
FCA098	A2	1,3	--	8	Leo098	92-112	0.63	8
FCA126	B1	1,2,3,4,5,6,7,8,9	≤0.78	8	Leo126	87-149	0.73	9
FCA224	A3	1,2,3,6	0.82	10	Leo224	78-96	0.67	7
FCA230	B3	1,3,6,7	0.72	10	Leo230	76-90	0.83	8
FCA247	C1	1,2,3,7	--	8	Leo247	114-132	0.83	8
FCA281	E1	1,3	--	12	Leo281	207-247	0.63	12
FCA391	B3	1,6,8	≤0.75	8	Leo391	170-198	0.70	8
FCA506	F2	6,8,9	≤0.83	14	Leo506	170-227	0.80	8

MATERIALS AND METHODS

Candidate microsatellites were chosen based on the number of previous lion studies that used the marker and the level of heterozygosity and number of alleles as determined by those previous studies (Table 2.1). Three lion samples (Sample ID: 2011000254, 2011000387 and 2011000446) were used to design the new STR primers. These samples have proven to have

good amplification success and were used as positive controls in another study[91]. PCR amplification was done using domestic cat primers[52] for the candidate microsatellites with the KAPA Biosystems KAPA2G™ Robust HotStart PCR Kit according to manufacturer's instructions using Buffer A. To ensure amplification and to identify the optimal PCR product for use in cloning, the annealing temperature was run on a gradient across the plate using VeriFlex™ 96-Well Thermal Cycler (Applied Biosystems) at 54–64°C by increments of 2°C. PCR product was visualized on a 1.5% agarose gel. PCR product that produced the brightest band and appeared to have the least amount of non-specific binding was chosen for cloning. Cloning was done using the TOPO® TA Cloning® Kit for Sequencing (Invitrogen) according to manufacturer's instructions. Each microsatellite was cloned a minimum of three times. Clones were sequenced on an Applied Biosystems 3130xl Genetic Analyzer then manually edited and aligned using SEQUENCHER v4.8 (Gene Codes Corporation). Consensus sequences were made from the aligned cloned sequences for each loci. Consensus sequences were aligned to their complementary domestic cat microsatellites in SEQUENCHER v4.8 to observe differences between species for each locus.

Lion FCA microsatellite consensus sequences were used to design primers closer to the repeat region and specific to the lion for each locus. These new markers are designated Leo STRs. We designed the primers using the CLC Genomic Workbench 8.5.1 (Qiagen) *Design Primers* tool. Parameters for primer design included a length of 18-22 bp, CG-content of 40-60%, and melting temperature of approximately 55°C. There is no standardized length for miniSTRs[86–88]. For this study, we define a miniSTR to be a redesigned STR less than 150 bp in size.

Table 2.2. Cloning and miniSTR design results by locus. Length for consensus sequences of clones is given for each locus (FCA Length). Length of the miniSTR (Leo Length) is used as the predicted length for the miniSTR with the new Leo forward and reverse primer sequences. T_a is the optimal annealing temperature determined during PCR. Fluorescent dye and mix are also noted. FCA primer sequences can be found in Menotti-Raymond et al, 1999. (Reprinted with permission from Curry & Derr, 2019)

	FCA		Leo					
Locus	Length	T_a (ºC)	Length	T_a (ºC)	Leo Forward Primer	Leo Reverse Primer	Mix	Dye
006	186	56	108	55	GACTTCTGCCTTCTTGTG	GTAGAATCGGTGTCCTTT	1	VIC
008	142	60	136	55	GTAAATTTCTGAGCTGGC	CAGACTGTTCTGGGTATGGT	2	NED
026	--	60	--	--	--	--	--	--
031	246	60	201	55	CAGGGACCTTAGTTAGATT	CCTTGCCTTTCTTAGTTATC	3	6-FAM
045	132	60	86	55	GCCAACTACCAAACAACA	GTATGAGCATCTCTGYGT	1	6-FAM
077	146	60	107	55	GAGATGTGAAAGTTGAAAGG	ACCAGTGTGATAAGAAAGAC	2	VIC
085	120	60	101	55	GCTCCCAGAATTTCTTATCT	TGCACTGGACAAGGATGG	2	6-FAM
091	136	56	--	--	--	--	--	--
096	--	48	--	--	--	--	--	--
098	117	60	115	55	TAGTCACAGCGCACATGC	CAGCCAGAATAAACCTCCA	4	VIC
126	139	62	132	55	ATACCCTGAATGCTCCAA	CTATCCTTGCTGGCTGAA	1	NED
224	160	56	93	55	CTTACACATGCACTCTCTC	CAGAGTTGTATGAAAGGGAC	3	VIC
230	94	56	88	55	ACAACAGGCAAAAGGGAA	AAGAATGGACTTGGGAAATGG	4	NED
247	147	60	126	55	AAGATTTACCCAGTTGCC	TGTCTGCTAGAGATGACCAA	3	NED
281	234	62	217	55	GTGGAGATGATGTGGATG	TTGGTTTCTCTCCCTACC	1	6-FAM
391	222	56	195	55	TCCTCAAACCAGTTCTTCC	GCCTTCTAACTTCCTTGC	2	6-FAM
506	216	56	200	55	GTGTAAGTTTAGGCGAGT	AATGACACCAAGCTGTTGTCC	4	6-FAM

Table 2.3. Sample information including DNA quality and quantity. DNA is assigned to four categories: good quality, DNA intact, high quantity (GIH), previously problematic, some DNA fragmentation, high quantity (PFH), low quality, DNA fragmented, high quantity (LFH), or likely degraded, DNA highly fragmented, low quantity (DFL). Quantity was measured by PCR concentration (ng/μL). The sample type, sex, geographical origin, and date the sample was collected are also given. *Samples used for miniSTR design. (Reprinted with permission from Curry & Derr, 2019)

Sample ID	Quality	Type	PCR	Sex	Origin	Date
2011000254*	GIH	Hide	20	M	Zambia - Kafue	2008
2011000263	PFH	Hide	20	M	Zambia - Luangwa Valley	2008
2011000282	LFH	Hide	20	M	Zambia - Luangwa Valley	1984
2011000304	PFH	Hide	20	M	Zambia - Luangwa Valley	2006
2011000326	PFH	Hide	20	M	Zambia - Kafue	2006
2011000330	PFH	Hide	20	M	Zambia - Luangwa Valley	2007
2011000387*	GIH	Hide	20	M	Zambia - Corridor	2011
2011000391	GIH	Hide	20	M	Zambia - Kafue	2011
2011000409	GIH	Hide	20	M	Zambia - Kafue	2011
2011000411	GIH	Hide	20	M	Zambia - Corridor	2011
2011000414	GIH	Hide	20	M	Zambia - Luangwa Valley	2009
2011000415	PFH	Hide	20	M	Zambia - Luangwa Valley	2009
2011000446*	GIH	Hide	20	F	Zambia - Lower Zambezi	2009
2011000472	GIH	Hide	20	M	Zambia - Luangwa Valley	2010
2011000706	GIH	Biopsy Tissue	20	F	Zambia - Luangwa Valley	2008
2011000710	PFH	Biopsy Tissue	20	F	Zambia - Luangwa Valley	2008
2011000750	GIH	Biopsy Tissue	20	M	Zambia - Kafue	2009
2011000783	GIH	Biopsy Tissue	20	M	Zambia - Kafue	2010
2011000814	GIH	Hide	20	M	Zambia - Kafue	2012
2011000838	GIH	Hide	20	M	Zambia - Luangwa Valley	2012
2011000847	GIH	Biopsy Tissue	20	M	Zambia - Kafue	2012
2011000854	GIH	Biopsy Tissue	20	F	Zambia - Kafue	2012
2011000855	GIH	Biopsy Tissue	20	F	Zambia - Kafue	2012
TLS	PFH	Bone - Skull	20	M	Tanzania	2009
AMNH 52081	DFL	Tooth	5	F	Democratic Republic of the Congo	1912
CM 5899	DFL	Turbinate	15	F	Tanzania	1927
FMNH 31121	DFL	Dried Tissue	1	M	India	1929
KU 105217	DFL	Bone Fragment	5	F	Tanzania	1920
LACM 51296	DFL	Bone Fragment	15	M	Kenya	1922
YPM 009570	DFL	Dried Tissue	15	M	Kenya	1931

The forward primer for each Leo STR was labeled with a fluorescent dye according to the predicted fragment length to allow for multiplexing (Table 2.2). PCR amplification was optimized for each marker individually using the KAPA Biosystems KAPA2G™ Robust HotStart PCR Kit. PCR was done using a touchdown technique to reduce stuttering [80]. The Leo STRs were multiplexed into four mixes (Table 2.2) and further optimized for genotyping. Detailed PCR and multiplex procedures for each mix can be found in Appendix A.2.a-c. PCR product was analyzed on a Applied Biosystems 3130xl Genetic Analyzer and genotypes were visualized and scored in STRand[92].

We tested the efficiency of the Leo STRs by fragment analysis of DNA from 30 lions (Table 2.3). DNA samples with SampleID: 2011000*** were chosen from an existing study[91]. Samples with a range of mitochondrial (mtDNA) haplotypes were chosen to capture the widest range of diversity. The remaining samples were selected from a collection of museum samples. DNA from these samples was extracted from various sample types (tissue, bone, hide) of different ages (collection dates ranged from 1912 to 2012) resulting in a range of DNA quality (highly fragmented to intact) and quantity (1-20 ng/μL) to demonstrate the versatility of the new primers.

For the museum samples, DNA was extracted from dried tissue using the DNeasy Blood and Tissue kit (Qiagen) following manufacturer's instructions for purification of total DNA from animal tissues. DNA was extracted from skeletal remains using protocols derived from Ambers *et al.*[93]. Detailed extraction protocols for this procedure, including handling, preparation, and processing of samples, can be found in Appendix A.1.c.

Processing of samples took place in a PCR workstation with dedicated equipment for work with lion samples. All work surfaces and equipment were thoroughly cleaned and exposed

to UV light before use. Consumables were sterilized by autoclave and exposed to UV light before use[94,95].

Quality and quantity classification for each sample can be found in Table 2.3. DNA quantity was measured by NanoDrop® (Thermo Scientific). High quantity DNA was diluted to a 20 ng/µL PCR concentration (**H). Lower quantity extractions (**L) were used undiluted. DNA quality was measured by visualization on a 2% agarose gel. Samples with a solid band of high molecular weight were marked as being "intact" (*I*) and "good quality" (G**). Smears were marked as being "fragmented" (*F*), with lower molecular weight smears (<1000 bp) additionally marked as "degraded" (D**). Samples that had previous difficulty with amplification [91], likely due to some fragmentation, were marked as "previously problematic" (P**).

PCR was done in a separate room from sample processing[94]. For PCR amplification, we used a multiple tubes approach[96,97]. Each sample was amplified and genotyped a minimum of two times for GIH and PFH samples and four times for LFH and DFL samples (Table 2.3). If there appeared to be any inconsistencies between allele calls, the sample was amplified an additional two times to reduce allelic dropout or potential PCR artifacts. PCR protocols can be found in Appendix A.2.c. Sample ID: 2011000254 was used as a positive control to verify successful amplification and genotyping. To monitor the possible occurrence of contamination, a negative control of only PCR reagents was used and DNA from a bison served as another negative control to show amplification does not occur in an unrelated species.

PCR product was visualized and scored in STRand[92] (Figure 2.1) and a consensus genotype was determined. Number of alleles and observed heterozygosity were calculated by locus using GenAlEx 6.5[98].

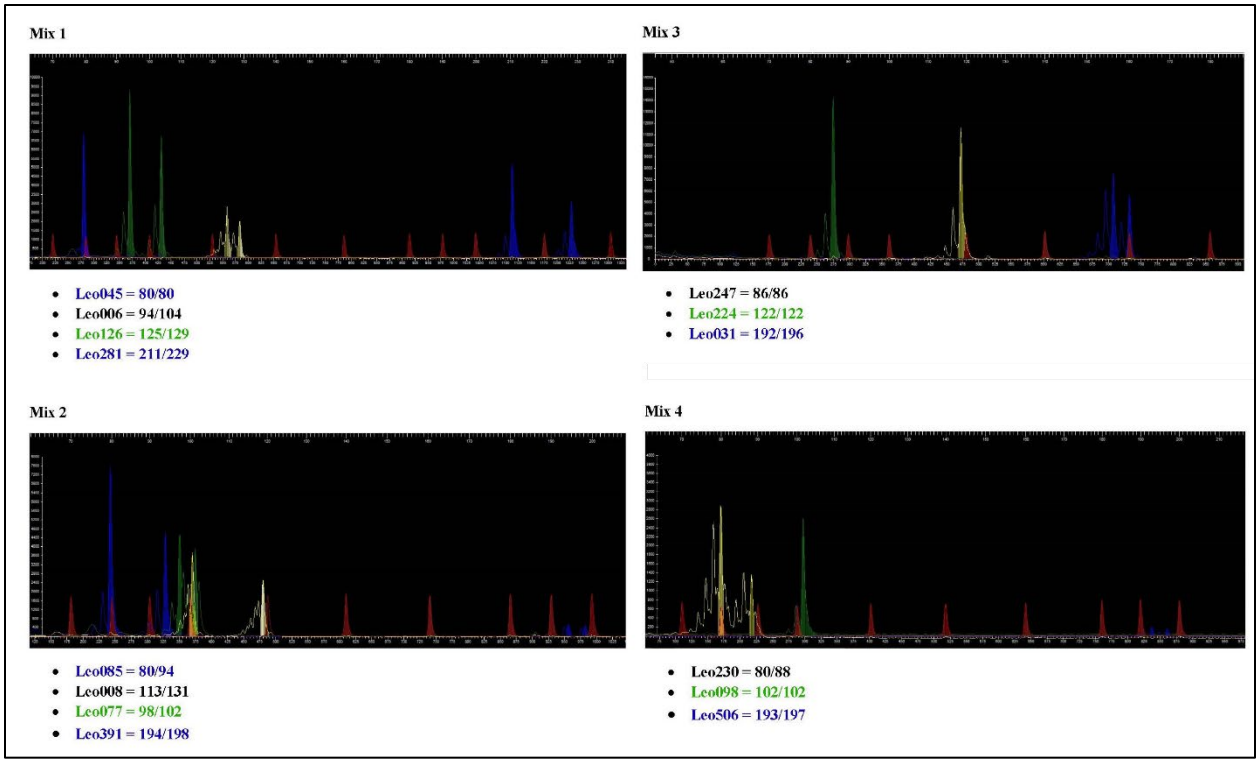

Figure 2.1. Screenshots of genotyping results in STRand for Sample 2011000254.

RESULTS

Seventeen candidate microsatellites were chosen (Table 2.1) from 107 previously used domestic cat microsatellite loci (Appendix C1). All 17 microsatellites were successfully amplified with the FCA domestic cat primers on the three samples chosen for redesign. FCA096 and FCA026 required the annealing temperature to be lowered to 44-54°C and 46-52°C, respectively, to produce a PCR product. PCR product from 16 of the 17 microsatellites produced clones that could be sequenced. We were unable to produce a clone for FCA096 despite multiple attempts and it was, therefore, eliminated.

Consensus sequences for 15 of the 16 cloned loci contained tandem repeats (GenBank Accession #s MH638611-MH638625). FCA026 was eliminated because the sequence did not

contain a repeat. Finally, FCA091 was eliminated because a primer pair could not be found within the sequence that matched the primer design criteria.

Table 2.4. Repeat motif of the lion and domestic cat with accession numbers of sequences used for alignment. (Reprinted with permission from Curry & Derr, 2019)

	Accession Numbers		Repeat Motifs	
Primer	**Lion**	**Domestic Cat**	**Lion**	**Domestic Cat**
FCA006	MH638611	AF130475	$(CA)_{20}$	$(CA)_{18}$
FCA008	MH638612	AF130476	$(CA)_{25}$	$(CA)_{26}$
FCA031	MH638613	AF130484	$(CA)_{10}TA(CA)_{13}$	$(CA)_{20}$
FCA045	MH638614	AF130489	$(CA)_{7}$	$(CA)_{15}$
FCA077	MH638615	AF130506	$(CA)_{20}$	$(CA)_{22}$
FCA085	MH638616	AF130513	$(CA)_{21}$	$(CA)_{19}$
FCA091	MH638617	AF130517	$(CA)_{19}$	$(CA)_{16}$
FCA098	MH638618	EU907355	$(CA)_{19}$	$(GT)_{17}$
FCA126	MH638619	AF130532	$(CT)_{12}$ GT$(CT)_{6}$ CAGA$(CA)_{23}$	$(CT)_{13}$ GT$(CT)_{5}$ CAGA$(CA)_{24}$
FCA224	MH638620	AF130574	$(CA)_{15}$	$(CA)_{13}$
FCA230	MH638621	AF130577	$(CA)_{20}$	$(GT)_{20}(AT)_{5}(GT)_{5}$
FCA247	MH638622	AF130583	$(CA)_{19}$	$(GT)_{19}$
FCA281	MH638623	EU907358	$(CA)_{14}$	$(CA)_{22}$
FCA391	MH638624	AF130624	$(CTAT)_{11}C(CCAT)_{12}$	$(GGAT)_{10}AGATGGATGG(GATA)_{12}$
FCA506	MH638625	AF130639	$(CA)_{25}$	$(GT)_{10}(GA)_{18}$

FCA microsatellite sequences for the lion were compared to the domestic cat revealing sequence differences including insertion and deletion events, and SNPs from transitions and transversions (Figure 2.2). In comparing sequences of FCA loci for the lion to that of the domestic cat (Figure 2.2 A and B), FCA085, FCA126, and FCA247 did not show any differences between the domestic cat and lion. The amount of differences between the domestic cat and the lion varied greatly across the rest of the loci with most occurring in the flanking sequence (Figure 2.2 A and B) and as differences within repeats (Table 2.4). FCA031 had a deletion in the

forward primer binding site in the lion. There was a transition between the lion and domestic cat in the FCA281 reverse primer binding site and FCA045 had a SNP within the reverse primer binding site in the lion but not the domestic cat.

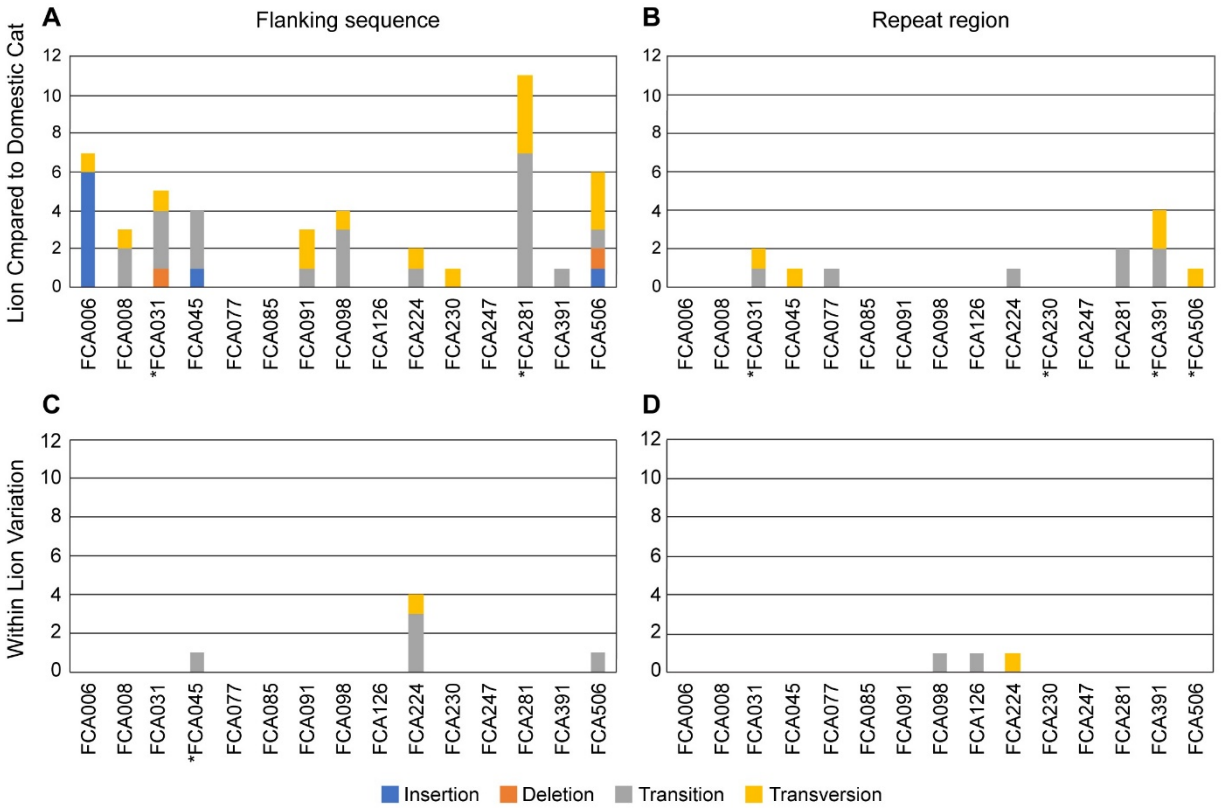

Figure 2.2. Single base differences in microsatellite sequences of lion compared to domestic cat (A and B) and within lion sequence variation (C and D). Sequence accession numbers can be found in Table 4. Differences include inversions, deletions, transitions, and transversions (see legend) in the flanking sequence (A and C) and repeat region (B and D). Within lion sequence variation are SNPs that are a result of lion sequences being a consensus generated by three lions. Lion specific SNPs differ from the domestic cat sequence at only one of the two variations. Loci with an asterisk (*) have a SNP in the primer binding site in the flanking sequence or a change of repeat motif in the repeat region. Differences caused by variation in repeat length or change of motif are not included here and can be found in Table 2.4. (Reprinted with permission from Curry & Derr, 2019)

Loci FCA045, FCA098, FCA126, FCA224, and FCA506 had SNP variation within the lion (Figure 2.2 C and D). Even with the redesigned Leo STRs, SNPs were present within the repeat of Leo098, Leo126 and Leo224. Leo045 had the SNP encoded within the forward primer but could not be designed to exclude the location. The Leo045 forward primer, therefore, included a Y (Table 2.2), IUPAC code for incompletely specified nucleic acids of a C or T[99]. Leo224 and Leo506 both had a SNP in the flanking sequence within a few bases of the repeat that could not be designed out.

We designed primers for 14 Leo STRs (Table 2.2), ten of which qualify as miniSTRs (Table 2.1). The four remaining STRs (Leo031, Leo281, Leo391, and Leo506) were still included although they could not be designed smaller than 150 bp. Fragment size was not shortened more than a few base pairs for some loci (i.e. 098 and 126). Although fragment size could not be changed, primers for these loci were still redesigned to improve specificity, as the FCA primer pairs did not fall within our primer design criteria.

The Leo STRs were optimized for individual and multiplex PCR and genotyping in four mixes (Table 2.5; Appendix C2). Mix 4 consisted of Leo098, Leo230 and Leo506. These three loci required separate PCRs. Primers for Leo098 were very efficient and outcompeted all other primers during PCR, even at low concentrations. Leo230 and Leo506 primers each produced a product when amplified individually but failed to produce a product when paired with other loci during PCR. Multiple primer sets were designed for these loci with similar multiplexing results. Individually amplified PCR product from these loci were pooled and genotyped together on the genetic analyzer.

All 30 test samples displayed 100% amplification success (Table 2.5) and loci had levels of heterozygosity comparable to those reported in previous studies (Table 2.1).

Table 2.5. Leo STR Allele Calls of 30 Test Samples. (Reprinted with permission from Curry & Derr, 2019)

	Mix 1								Mix 2								Mix 3						Mix 4					
Sample	Leo006		Leo045		Leo126		Leo281		Leo008		Leo077		Leo085		Leo391		Leo031		Leo224		Leo247		Leo230		Leo098		Leo506	
2011000254	94	104	80	80	125	129	211	229	113	131	98	102	80	94	194	198	192	196	86	86	122	122	80	88	102	102	193	197
2011000263	108	122	80	102	127	127	213	231	111	131	98	104	76	80	170	174	196	196	78	92	122	126	80	88	102	104	173	195
2011000282	122	124	80	80	125	127	213	213	113	125	98	106	80	80	174	178	188	200	86	92	126	132	76	76	102	102	173	191
2011000304	82	82	80	80	125	129	213	231	111	111	98	112	80	80	174	174	196	196	86	86	116	126	80	80	102	106	193	193
2011000326	108	122	80	94	105	129	213	231	113	123	102	104	76	94	190	194	196	196	78	86	122	126	76	86	92	104	191	191
2011000330	104	128	80	80	105	127	213	213	113	127	98	104	80	94	174	174	188	196	86	92	114	130	76	80	102	106	193	193
2011000387	104	104	80	80	131	143	213	217	127	127	102	104	80	80	174	190	196	196	78	86	114	116	78	82	104	108	191	193
2011000391	94	108	80	80	125	129	213	213	113	131	104	110	76	94	174	174	196	196	78	86	114	116	82	88	102	104	191	193
2011000409	94	108	80	80	125	129	213	213	113	131	98	102	76	80	174	174	196	196	86	94	114	116	76	86	102	104	191	193
2011000411	104	124	80	80	125	125	223	223	111	133	102	106	80	92	174	174	188	194	86	86	114	132	80	88	94	108	171	191
2011000414	108	124	80	80	127	127	213	223	111	127	104	104	80	80	178	178	196	196	78	86	114	128	80	88	102	102	191	193
2011000415	108	124	80	102	127	129	213	223	111	127	98	102	80	80	174	178	188	196	86	94	114	128	80	90	102	106	191	193
2011000446	104	108	80	80	125	127	213	213	127	131	102	104	76	80	174	178	196	196	86	92	122	128	78	90	102	106	191	193
2011000472	108	108	80	80	125	127	213	223	111	113	98	102	80	80	170	174	196	196	78	78	124	128	80	88	102	106	171	195
2011000706	94	104	80	96	125	131	213	223	111	113	104	104	80	80	170	194	190	196	78	78	116	128	80	88	102	106	191	191
2011000710	108	108	80	80	105	105	213	213	113	127	104	102	74	74	186	198	196	196	78	78	126	128	76	88	102	102	173	191
2011000750	122	124	80	80	125	129	213	213	113	131	98	102	74	76	174	198	188	196	86	86	114	116	80	88	102	104	171	171
2011000783	94	122	80	80	105	105	213	215	113	127	102	104	74	80	186	198	192	196	78	94	114	128	80	88	108	108	193	195
2011000814	104	104	80	80	125	127	213	217	131	131	104	108	76	80	190	198	192	196	78	92	128	128	76	88	102	106	171	193
2011000838	108	124	80	80	125	125	213	243	113	125	102	108	76	76	174	174	196	196	78	86	122	128	76	76	102	102	191	195
2011000847	104	124	80	80	105	127	213	223	127	127	102	104	74	80	186	186	192	196	78	78	114	116	88	88	102	104	173	181
2011000854	94	108	80	80	125	131	213	213	127	131	106	110	76	80	174	182	192	196	90	92	116	126	76	88	102	104	171	195
2011000855	108	122	80	80	125	125	213	247	111	131	104	106	80	94	174	186	196	196	86	94	122	122	76	90	92	102	193	193
AMNH 52081	110	130	80	80	125	149	223	229	111	131	100	110	86	92	178	182	194	196	92	96	122	124	76	86	92	112	171	175
CM 5899	90	112	80	94	107	127	207	213	111	127	102	110	80	92	186	198	186	196	86	96	126	130	86	90	102	102	173	175
FMNH 31121	94	94	80	80	129	129	223	223	113	125	106	106	80	80	186	186	192	192	86	92	130	130	78	78	106	106	171	175
KU 105217	82	130	80	80	127	129	221	225	111	129	102	104	80	92	170	186	186	196	92	96	116	130	78	84	100	100	187	195
LACM 51296	90	122	80	110	87	105	223	229	113	113	104	106	94	94	174	190	196	196	94	94	128	128	80	90	104	104	171	175
TLS	124	124	80	100	129	143	213	223	113	125	104	108	76	80	174	182	196	196	88	96	114	116	76	88	102	102	171	191
YPM 009570	94	124	80	94	127	143	213	235	111	129	104	110	72	76	182	194	186	186	92	92	122	128	80	90	102	106	173	175
# success	30		30		30		30		30		30		30		30		30		30		30		30		30		30	
A	11		6		9		12		8		8		7		8		7		7		8		8		8		8	

DISCUSSION

Redesigned Leo miniSTRs improved upon FCA microsatellites for use in lions by reducing issues from cross-species amplification through having decreased fragment size, lion specific primers, and equivalent annealing temperatures (Table 2.2). Fourteen of the 17 candidate microsatellite loci were successfully redesigned as Leo STRs that exhibited 100% amplification success when tested on DNA of 30 lions with varying DNA quality and quantity.

Three of the 17 candidate microsatellite loci could not be redesigned. FCA026 is a popular locus, used in nine out of twelve studies, but we were unable to verify that it is, in fact, a microsatellite because the fragment produced during cloning did not contain a tandem repeat. FCA096 amplified but was unable to be cloned, therefore, we could not determine if it is a microsatellite. The annealing temperature was lowered for both FCA026 and FCA096 compared to the other loci (Table 2.2). Lowering annealing temperature can increase amplification success[100] but temperatures below 50°C increase the chances for non-specific binding[101]. This may have contributed to our inability to find the desired sequences from these two loci. FCA091 was successfully cloned and sequenced but we could not find a suitable primer pair based on our criteria.

The amount of differences varied greatly across loci. Most differences occur in the flanking sequence. Point mutations in the flanking sequence, especially within the primer binding site, can inhibit annealing[102]. Changes in the primer-annealing region can affect binding and increase the chance for null alleles[60], such as with FCA031, FCA045, and FCA281. But differences that result in sequence length differences or changes in GC content can affect the melting temperature or secondary structure[79,100]. The repeat in FCA230 and FCA506 for the lion was a simple CA-dinucleotide motif while the domestic cat motif changes mid-repeat creating a

complex combination of 2-3 motifs. Studies have found there to be no association between differences in repeat motif or structure[67,100] unless those differences result in a notable change to the repeat length[100] or GC content[79]. We suggest using the improved Leo primers for these 14 loci, however, if fragment length and equivalent annealing temperature for multiplexing are not a concern, FCA primers could be used for loci 085, 126, and 247, which did not show any differences between the domestic cat and lion.

SNP variation was also found within the Leo STRs (Figure 2.2 C and D). We attempted to eliminate this variation with primer redesign but could not in all cases. STR redesign could only reduce the number of SNPs for locus 224, from five to two SNPs. This SNP variation, called detectable homoplasy[60], cannot be seen via fragment length analysis, only by sequencing. Therefore, total variation in these loci may be underestimated. However, homoplasy only introduces minimal bias[103] so we believe diversity determined by sequence length variation alone in these loci is still informative.

Previous mtDNA research in Zambia show the lion population is highly diverse[91]. Therefore, we considered results from the 30 test samples to be comparable if they had high heterozygosity and were within 1-2 alleles reported in previous studies. Leo031 and Leo045 had a comparable number of alleles but had lower heterozygosity than anticipated. While using miniSTRs reduces the possibility of null alleles and allelic dropout[86,87], these could still be a possible cause for lowered heterozygosity. Based on these results, Leo031 and Leo045 are suitable loci but should be used with caution. All other loci are considered within a comparable range and are recommended for use. Further analysis of more samples from Zambia using Leo STRs[104] also had heterozygosity and number of alleles comparable to these test samples.

SNPs from next-generation sequencing are growing in popularity[105]. SNPs have been used to infer lion phylogenies[106], but these analyses can be expensive. Microsatellites have a higher mutation rate and higher information content than SNPs, requiring fewer markers for equivalent power, making them more cost effective for non-model species[107–109] for forensics and population studies.

For lions, miniSTRs specific to the species will benefit conservation genetic research and forensics by providing methodology that offers greater amplification success with degraded samples, such as from non-invasive sampling, forensic samples, or historical samples from museums. By using miniSTRs that are based off previously used microsatellites, data can be calibrated and standardized to be combined with and compared to previous genetic knowledge.

CHAPTER III

MITOCHONDRIAL HAPLOTYPE DIVERSITY IN ZAMBIAN LIONS: BRIDGING A GAP IN THE BIOGEOGRAPHY OF AN ICONIC SPECIES[2]

INTRODUCTION

In Zambia, the African lion (*Panthera leo*) is broadly but irregularly distributed across approximately 167,000 km^2 of managed habitats comprised of national parks (NP) and game management areas (GMAs). Recent estimates propose the total number of wild lions in Zambia to be between 1000 to 2000 individuals[2,7,19,34]. The largest numbers are reported from the Luangwa Valley ecosystem located in the eastern part of the country (density of 2.0[110] to 4.0[34] lions per 100 km2), with the second largest concentration of lions located in the Kafue ecosystem in the west (density of 1.5[110] to 1.83[111] lions per 100 km^2). Only recently have Zambia's lions come under more intensive scientific investigation[15,34,110–112]. A study utilizing nuclear microsatellite markers and the Cytochrome-b mitochondrial marker established Zambian lions in a larger-scale genetic perspective[15] and showed that Zambian lions exhibited some intermixing of genetic profiles found in eastern and southern Africa. Despite these intriguing findings and the importance of the geographic location of this range state in relation to other countries where lions occur, there remains little information regarding genetic diversity or population sub-structure of lions within Zambia.

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[2] Reprinted with permission from "Mitochondrial Haplotype Diversity in Zambian Lions: Bridging a Gap in the Biogeography of an Iconic Species" by Curry, C. J., White, P. A. & Derr, J. N. PLoS One 10, e0143827 (2015).

For this study, we calculated the extent of genetic diversity and matrilineal distribution in Zambian lion populations through the analysis of the 12S to 16S mitochondrial genes (mtDNA) of 165 lions found in five main areas in Zambia (Figure 3.1). Through extensive sampling of individuals from NPs as well as GMAs, we achieve a finer resolution image of population genetics of lions found throughout Zambia.

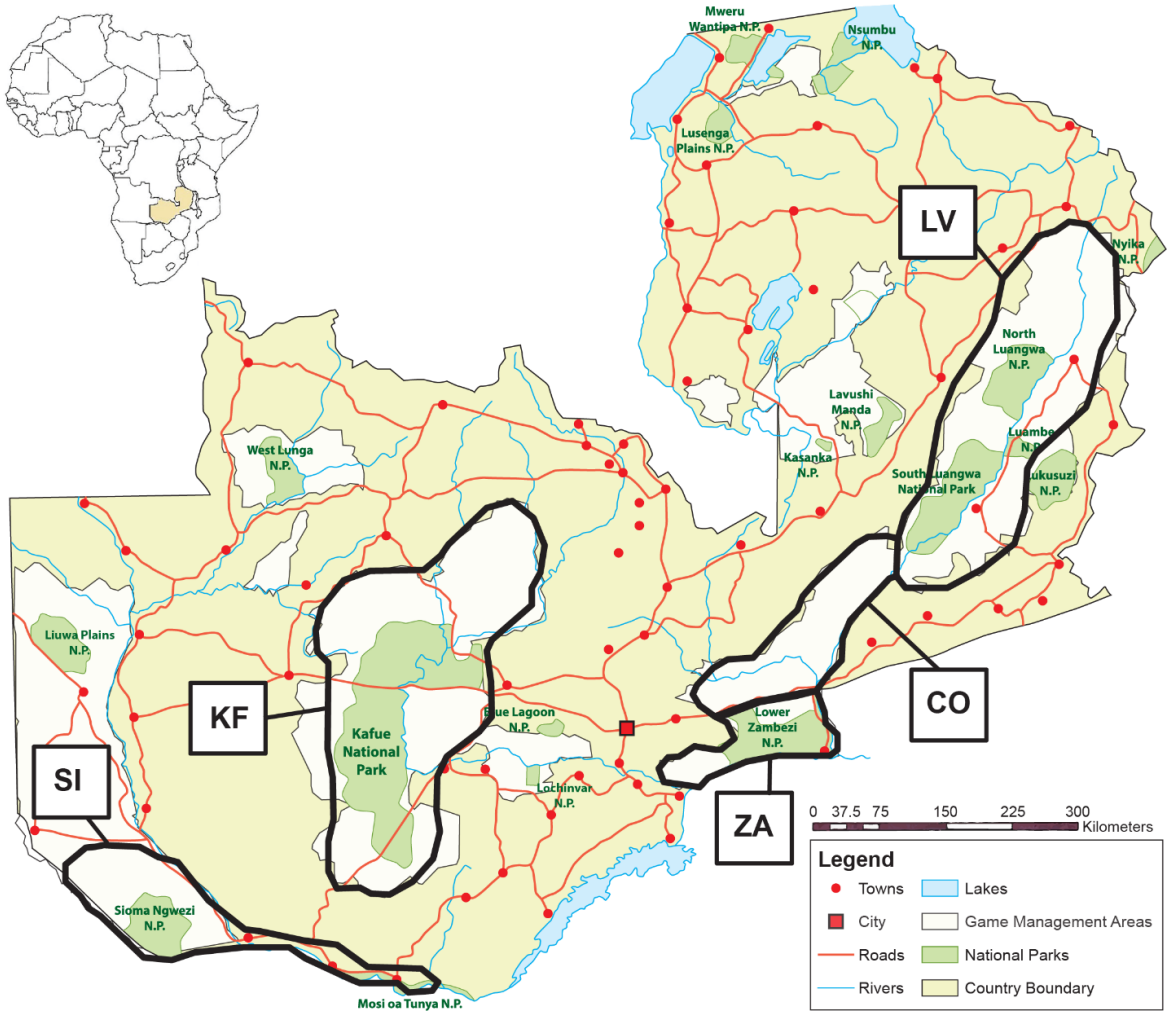

Figure 3.1. Map of Zambia showing the five main areas sampled: LV (Luangwa Valley); CO (Corridor); ZA (Lower Zambezi); KF (Kafue); and SI (Sioma Ngwezi). Eastern region consists of LV, CO and ZA. Western region consists of KF and SI. More detailed location information for each sample is available in Appendix B.1.a. (Reprinted with permission from Curry *et al*. 2015)

Mitochondrial DNA (mtDNA) is maternally inherited and has a relatively fast mutation rate that results in significant variation in mtDNA sequences. Lion prides typically consist of 2-18 related females born to that pride with 1-7 males who migrate into the pride from elsewhere[9]. When male dispersal is high while female dispersal is low, as is true in African lions, it is possible to detect geographic structure through the use of mtDNA[113].

The 12S and 16S genes of the mtDNA encode ribosomal RNAs (rRNA) necessary for the translation of messenger RNAs into mitochondrial proteins. The 12S and 16S genes are more conserved than the protein-coding genes of mtDNA[102] with 12S slightly more conserved than 16S[114]. Due to this conservation, haplotype diversity represents a deeper, more historic level of diversity within the population. Levels of genetic diversity are directly proportional to a species' ability to adapt, survive and thrive. Therefore, loss of genetic diversity is detrimental to overall population health and long-term survival because it decreases the population's potential to adjust to environmental changes or perturbations. We consider our findings at the population and sub-population scale and discuss potential ramifications of genetic sub-structure for lion management and conservation.

MATERIALS AND METHODS

Sample Collection

African lion DNA samples (hair, skin, bone and/or tissue) were collected during research conducted by the Zambia Lion Project (ZLP) while operating in partnership with the Zambia Wildlife Authority (Research/Employment Permit No. #008872). Samples were collected between the years of 2004-2012 from dried skins of trophy hunted lions, biopsy darting of free-ranging live lions, and tissue or skin samples of "problem" lions killed by the Zambia Wildlife

Authority. The Zambia Wildlife Authority includes a research division and veterinary division that reviews all proposed studies, including animal care and use protocols, and approves studies only after they have met department standards. For this study, in addition to the research division's review of the proposal, Zambia Wildlife Authority's chief veterinarian reviewed the sampling protocol and examined the veterinary projector, cartridges, and biopsy darts prior to approval. Review of the proposal included interviews with ZLP's Principal Investigator, P.A. White to discuss in detail the sampling protocol and field testing of the biopsy darting equipment.

Lion skin was obtained by collecting a small (1x1cm) snip of dried skin with hair attached and storing it in individually labeled paper envelopes. Trophy lion skins were from male animals previously sport hunted under strict permitting by Zambia Wildlife Authority and in accordance with national hunting regulations. Problem lion skins were male and female animals destroyed by Zambia Wildlife Authority. No lions were sacrificed specifically for the Zambia Lion Project. Where skins were not available to sample, small (1x2cm) fragments of turbinate bones were collected from the nasal passages of cleaned skulls.

Biopsy tissue samples were collected from live lions using a 4x scoped Pneu-dart Model 389 cartridge fired veterinary projector that propelled a 3cc Pneu-dart biopsy dart specially designed for use on African lion (Pneu-dart, Williamsport, PA). Darts were fired from a range of 15-60m using green CCI power loads. Prior to firing a dart, a rangefinder was used to gauge distance to the lion. A 5-position pressure control dial on the projector allowed the power of the dart to be safely controlled over a broad range of darting distances. The tip of the biopsy dart contained a cutting farrel that upon impact punched a plug 3mm in diameter x 5mm in length from the lion's shoulder or rump. The dart, which contained no drugs, bounced off immediately

following impact retaining the tissue plug on a barb inside the farrel. Both male and female lions older than approximately one year of age were biopsy darted. Cubs younger than one year old were not sampled.

Tissue samples were immediately removed from the dart using sterile tweezers and placed into individually labeled vials containing 95% EtOH. Skin and tissue samples were stored in Zambia at room temperature until being transferred to a USA laboratory for analysis. Samples were collected and imported in full compliance with specific legal national and international permitting requirements. Samples were imported to the USA under CITES permits numbers #25393 and #30208.

DNA samples were obtained from both male and female lions throughout Zambia's National Parks and GMAs making this dataset representative of Zambia's countrywide lion population. A continuous sequence of the 12S-16S genes (1880-1882 base pairs) was analyzed from sequences successfully amplified from 165 lions (119 males, 45 females, 1 unknown; Table 3.1) found in five main areas in Zambia (Figure 3.1). These areas include five national parks (North Luangwa, South, Luangwa, Lower Zambezi, Kafue and Sioma Ngwezi) and twenty-nine GMAs.

Table 3.1. Number of males (♂), females (♀), and unknown sex (?) for each haplotype for all areas sampled in Zambia along with the haplotype frequencies. Haplotypes H1, H9 and H11 were previously described by Antunes *et al*.[38] Haplotypes Z1-Z5 are novel. (Reprinted with permission from Curry *et al*. 2015)

	Eastern Region									*Western Region*								
	LV			CO			ZA			KF			SI					
Haplotype	♀	♂	?	♀	♂	?	♀	♂	?	♀	♂	?	♀	♂	?	n	*f*	s.d.
H1										0	1	0				1	0.0061	0.0061
H9	0	1	0							18	41	0	0	1	0	61	0.3697	0.0377
H11	6	13	0	3	5	0	1	0	0	0	1	0				29	0.1758	0.0297
Z1	7	32	1	0	6	0	1	0	0	0	4	0				51	0.3091	0.0361
Z2							0	1	0							1	0.0061	0.0061
Z3										5	10	0				15	0.0909	0.0224
Z4										0	2	0				2	0.0121	0.0085
Z5										4	1	0				5	0.0303	0.0134
n	13	46	1	3	11	0	2	1	0	27	60	0	0	1	0	165		
		60			14			3			87			1				
A		3			2			3			7			1		8		

n = sample size; for ♂, ♀ and? for each area and by area, haplotype and total.
A = number of haplotypes.
f = frequency.
s.d. = standard deviation.

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Molecular Analysis

To allow for a direct comparison with previously published data, we used the same maternal sequence (mtDNA) assessed by Antunes *et al*.[38] whose analysis did not include this region of Africa. The *Panthera* genus has a large 12.5 kb integration of mtDNA into the nuclear genome, or numt[56], which could be a potential source of error during analysis. False sequences of mtDNA/numt recombinants produced during PCR[57] can result in inaccurate levels of genetic diversity. To prevent potential numt amplification, mtDNA specific primers for the 12S-16S region designed by Antunes *et al*.[38] to prevent numt amplification were used.

DNA isolation, PCR and DNA sequencing and analysis were completed using standard laboratory techniques in the DNA Technologies Core Laboratory at Texas A&M University in College Station, TX (http://vetmed.tamu.edu/dnacore; details in Appendix A.1.a-b and A.2.d-e). PCR amplification was conducted using the KAPA Biosystems KAPA2G™ Robust HotStart

PCR Kit according to manufacturer's instructions. The cycling profile was as follows: initial denaturation at 95°C for 3 min, then denaturation, primer annealing and extension at 95°C for 15 s, 55°C for 15 s and 72°C for 45 s for 35 cycles, followed by a 1 min extension at 72°C. Samples were then cooled and held at 4°C until sequencing. PCR products were sequenced on an Applied Biosystems 3130xl Genetic Analyzer then aligned, manually edited and assigned a haplotype using SEQUENCHER v4.8[115].

Statistical Analysis

Genetic diversity calculations were implemented using Arlequin v3.5[116]. The number of polymorphic sites, gene diversity, nucleotide diversity and haplotype frequency estimations were calculated as a single population. Lions were divided into sub-populations and combined regionally for intra-population calculations of the coefficient of differentiation (F_{ST}) and hierarchical analyses of molecular variance (AMOVA). Pairwise differences (π) between and within populations were computed along with Nei's distance (d) through the use of conventional F-statistics.

Phylogenetic analysis included all haplotypes from Antunes *et al*.[38] (GENBANK Accession #s FJ151641-FJ151652) and novel haplotypes found in this study (GENBANK Accession #s KT164799-KT164803). The tree was rooted by the tiger (*Panthera tigris*) with a sequence from the complete mitogenome (GENBANK Accession #KJ508413) which was aligned to the lion sequences then trimmed to contain the same regions. Phylogenetic analysis was performed using Maximum Likelihood (ML) and Bayesian inference methods. ML analysis was then performed using Garli v2.01[117], RAxML[118], and PhyML[119]. A Bayesian analysis was conducted in Mr. Bayes[54,120] via Markov chain Monte Carlo (MCMC). Samples were drawn

every 1,000 steps over 50,000,000 MCMC steps. The first 10% were discarded as burn-in. Acceptable sampling and convergence to the stationary distribution were checked by inspection of traces using Tracer v1.5[121] and trees were visualized using FigTree v1.4.0[122]. In addition, a haplotype network was formed utilizing the median-joining option of Network v4.6.1.3[123].

RESULTS

When considered as one population, gene diversity throughout the population of lions in Zambia was high at 0.7319 +/- 0.0174. AMOVA analysis, run with each of the main areas within Zambia grouped regionally as an eastern [Luangwa Valley (LV), Corridor (CO) and Lower Zambezi (ZA)] and a western [Kafue (KF) and Sioma Ngwezi (SI)] sub-population (Figure 3.1), resulted in an F_{ST} of 0.47 (p-value<0.001) between regional sub-populations (Table 3.2). Within the eastern sub-population, F_{ST} calculated between areas was 0.05. F_{ST} was not calculated between areas within the western sub-population due to SI contributing only one sample that would have skewed the result. Gene diversity was equal but decreased slightly when the population was separated regionally (eastern at 0.5057 +/- 0.0575, western at 0.5014 +/- 0.0336).

Table 3.2. AMOVA results with F_{ST}. Percent variation is given among populations (Va) and within groups (Vb). The significance of differentiation within and among populations was tested by 1,000 permutations. (Reprinted with permission from Curry *et al*. 2015)

Source of Variation	d.f.	Sum of Squares	Variance Components	Percentage of Variation	p-value
Among Populations	1	18.966	0.22785 Va	47.50	<0.001
Within Populations	163	41.052	0.25185 Vb	52.50	<0.001
Total	164	60.018	0.47971		
Fixation Index	F_{ST}:	0.47499			

doi:10.1371/journal.pone.0143827.t002

Eight haplotypes were found; three haplotypes (H1, H9, H11) described by Antunes *et al.*[38] and five previously unreported haplotypes (Z1, Z2, Z3, Z4, Z5). The previously unreported haplotypes were regarded as true, novel haplotypes when they appeared two or more times. Haplotypes that appeared only once were verified through re-sequencing before being regarded as true, novel haplotypes. Of the five novel haplotypes, three were considered rare with frequencies below 5% (Table 3.1). Of the three previously described haplotypes, H1 and H9 were found in northern Botswana and Namibia while H11 was found throughout eastern Africa spanning from Uganda across the Serengeti to the Ngorongoro Crater in Tanzania as well as in southern Botswana and Kruger National Park in South Africa (Figure 3.2).

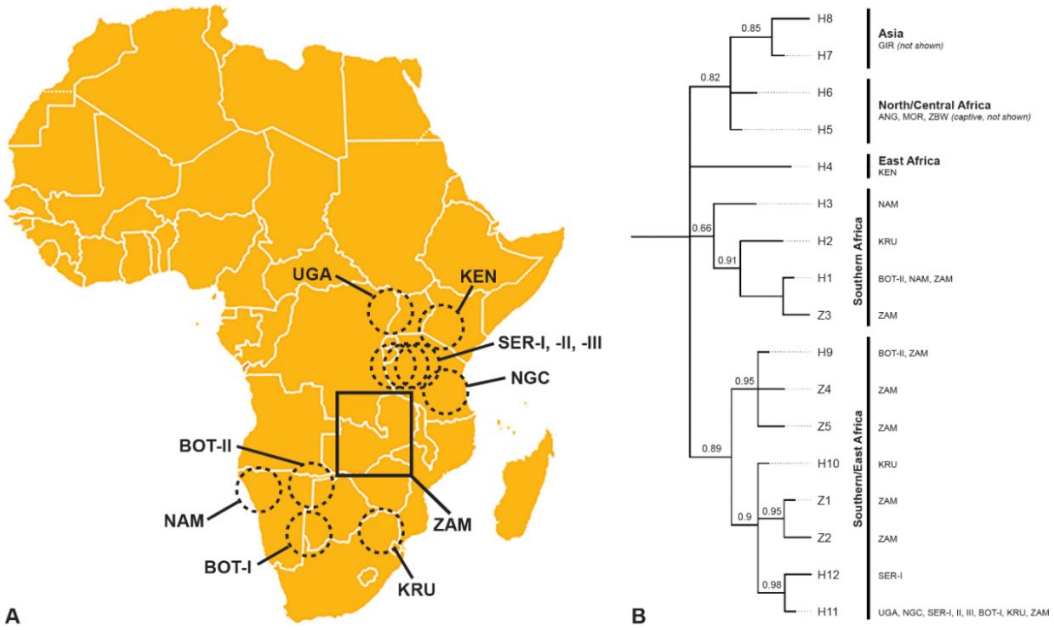


Figure 3.2. Geographic location of lion samples and phylogenetic relationship of 12S-16S. (A) Range-wide map of lions sampled. Circles indicate geographic locations for populations determined by Antunes *et al.*[38]. All locations aside from ZAM (Zambia) are from Antunes *et al.*[38]: UGA (Uganda); KEN (Kenya); SER (Serengeti NP); NGC (Ngorongoro Crater); KRU (Kruger NP); BOT-I (Southern Botswana and Kalahari); BOT-II (Northern Botswana); NAM (Namibia); GIR (India); ANG (Angola); ZBW (Zimbabwe); and MOR (Morocco). (B) Bayesian analysis with posterior probability values on the nodes. (Reprinted with permission from Curry *et al*. 2015)

Z1, found countrywide, differs from H10 by only one base pair. H10 is a haplotype seen only in Kruger National Park and is found with H2 (also seen only in Kruger National Park) and H11 (2 base pair differences from Z1, also countrywide). Haplotype Z2, which only appeared once, was verified through re-sequencing. Haplotype Z2 differs from haplotype Z1 by only one polymorphic site. This polymorphic site is also the only transversion, an adenine (purine) to a thymine (pyrimidine) substitution, seen at any polymorphic site between all haplotypes (as shown in Table 3.3, *position 1801*). The nucleotide differences by position and the number of base pair differences among haplotypes are shown in Table 3.3 and 3.4, respectively. Z3 has two insertions, similar to H1. Z3 was prevalent in KF and was the fourth most common haplotype overall with a frequency of 0.091. In contrast, H1 appeared only once (frequency=0.006) in a sample from KF but was found elsewhere in Northern Botswana and Namibia. Z4 and Z5, seen only in KF, each differ from H9, the predominant haplotype of KF (frequency=0.370), by only one base pair each.

Table 3.3. Nucleotide position for each polymorphic site. (Reprinted with permission from Curry *et al*. 2015)

	242	369	393	462	513	531	571	596	631	684	695	732	798	812	840	841	928	929	961	1039	1082	1140	1220	1247	1328	1387	1610	1629	1632	1646	1801
H1*	C	C	A	T	C	G	G	A	T	T	G	C	T	G	T	A	A	A	A	C	C	A	A	T	A	T	C	C	T	C	A
H2	•	•	•	•	•	A	•	•	•	•	•	•	•	•	C	•	•	-	•	•	•	•	•	C	•	•	•	T	•	•	•
H3	•	•	•	•	T	A	•	•	•	•	•	•	•	•	•	•	•	-	G	•	•	•	G	•	•	•	•	T	•	•	•
H4	•	•	•	C	•	A	•	•	•	•	A	T	C	•	•	•	-	-	G	•	T	•	•	•	•	•	T	T	•	T	•
H5	•	T	•	•	•	A	•	•	C	•	•	•	•	•	•	•	-	-	G	•	•	•	•	•	•	•	•	T	•	T	•
H6	•	T	•	•	•	A	•	•	C	•	•	•	•	A	•	•	-	-	G	•	•	•	•	•	•	•	•	T	•	T	•
H7	T	T	•	•	•	A	•	•	C	•	•	•	•	•	•	•	-	-	G	•	•	G	•	•	•	•	•	T	•	T	•
H8	T	T	•	•	•	A	•	•	•	C	•	•	•	•	•	•	-	-	G	•	•	G	•	•	•	•	•	T	•	T	•
H9	•	•	•	•	•	A	•	•	•	C	•	•	•	•	•	G	-	-	G	•	•	•	•	•	G	•	•	T	•	T	•
H10	•	•	•	•	•	A	•	•	•	C	•	•	•	•	•	•	-	-	G	•	•	•	•	•	G	C	•	T	•	T	•
H11	•	•	•	•	•	A	•	•	•	C	•	•	•	•	•	•	-	-	G	T	•	•	•	•	G	C	•	T	•	T	•
H12	•	•	•	•	•	A	A	•	•	C	•	•	•	•	•	•	-	-	G	T	•	•	•	•	G	C	•	T	•	T	•
Z1	•	•	•	•	•	A	•	•	•	C	•	•	•	•	•	•	-	-	G	•	•	•	•	•	G	C	•	T	C	T	•
Z2	•	•	•	•	•	A	•	•	•	C	•	•	•	•	•	•	-	-	G	•	•	•	•	•	G	C	•	T	C	T	T
Z3	•	•	•	•	•	•	•	•	•	•	A	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•
Z4	•	•	G	•	•	A	•	•	•	C	•	•	•	•	•	G	-	-	G	•	•	•	•	•	G	•	•	T	•	T	•
Z5	•	•	•	•	•	A	•	G	•	C	•	•	•	•	•	G	-	-	G	•	•	•	•	•	G	•	•	T	•	T	•

*All nucleotide polymorphisms in 12S-16S are shown for haplotype H1. For all other haplotypes, only nucleotides that differ from H1 are shown.
Haplotypes found in Zambia are in **bold**.

doi:10.1371/journal.pone.0143827.t003

Table 3.4. Polymorphic sites between all 12S-16S mitochondrial haplotypes. H1- H12 are haplotypes which were described by Antunes *et al* [13] and Z1-Z5 are novel haplotypes so far only found within Zambia.

H1	H2	H3	H4	H5	H6	H7	H8	H9	H10	H11	H12	Z1	Z2	Z3	Z4	Z5	
H1	5	6	12	8	9	10	10	9	9	10	11	10	11	1	10	10	H1
	H2	5	11	7	8	9	9	8	8	9	10	9	10	6	9	9	H2
		H3	10	6	7	8	8	7	7	8	9	8	9	7	8	8	H3
			H4	8	9	10	10	9	9	10	11	10	11	11	10	10	H4
				H5	1	2	4	5	5	6	7	6	7	9	6	6	H5
					H6	3	5	6	6	7	8	7	8	10	7	7	H6
						H7	2	7	7	8	9	8	9	11	8	8	H7
							H8	5	5	6	7	6	7	11	6	6	H8
								H9	2	3	4	3	4	10	1	1	H9
									H10	1	2	1	2	10	3	3	H10
										H11	1	2	3	11	4	4	H11
											H12	3	4	12	5	5	H12
												Z1	1	11	4	4	Z1
													Z2	12	5	5	Z2
														Z3	11	11	Z3
															Z4	2	Z4
																Z5	Z5

Average number of pairwise differences and Nei's distance (d) are shown in Figure 3.3. Nei's distance (d) and between population pairwise differences were highest between an eastern (LV, CO or ZA) and western area (KF or SI). While the ZA shows the highest level of within population pairwise differences (1.0), this is due to all three samples from this area having different haplotypes. Areas with higher sample sizes (KF, CO, LV) exhibit similar levels of within population pairwise differences (0.51, 0.53, 0.46, respectively).

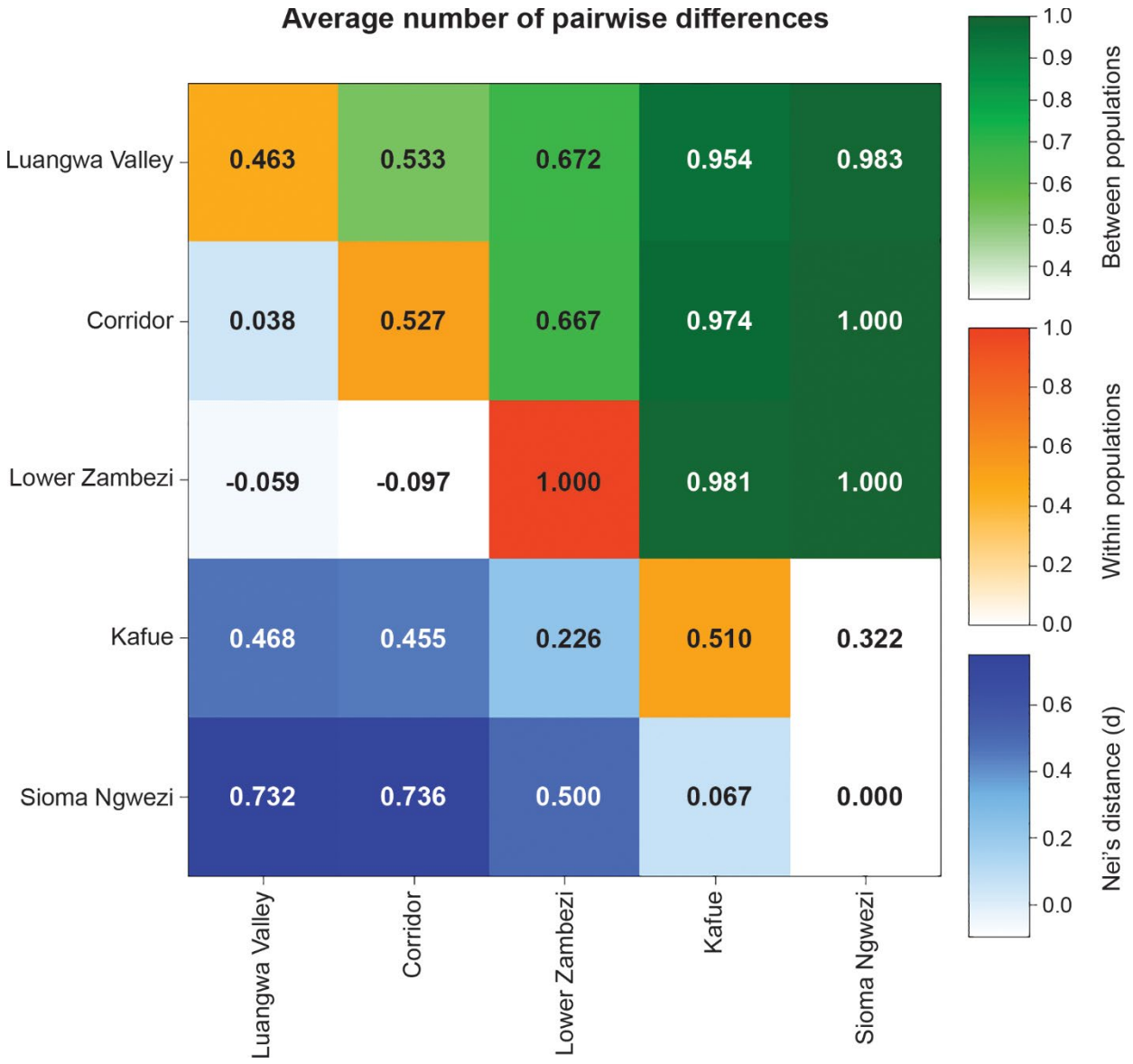


Figure 3.3. Nei's distance (d) and average number of pairwise differences within and between populations. (Reprinted with permission from Curry *et al*. 2015)

It is necessary to look at the occurrence of haplotypes range-wide and how haplotypes in neighboring areas compare to one another to understand the diversity present. A comparison of molecular diversity indices and nucleotide composition for haplotypes found within Zambia versus all haplotypes range-wide are shown in Table 3.5. Phylogenetic analysis was conducted

to bring the Zambian population into context with the entire range of the African lion. The Bayesian analysis is presented (Figure 3.2B) supported by posterior probability values of >60% for all nodes. While all trees resulted in similar clustering, Bayesian posterior probabilities offered stronger support than ML bootstrap values. Four regionally grouped clusters can be identified for *Panthera leo* – Asia/Central/Northern Africa, East Africa, Southern Africa and Southern/East Africa. The Southern/East Africa group consists of two branches, one containing south central Africa (Botswana and Zambia) and the other eastern Africa from South Africa northwards to Kenya. The same regional clusters could be found in the haplotype network (Figure 3.4).

Table 3.5. Molecular diversity indices and nucleotide composition. (Reprinted with permission from Curry *et al*. 2015)

***Within*:**	***Zambia***	***Range-wide***
Nucleotide Sites:	1882	1882
# of Haplotypes:	8	17
Polymorphic Sites:	16	31
Transitions:	13	28
Transversions:	1	1
Indels:	2	2
Nucleotide Composition		
C:	22.11%	22.07%
T:	22.67%	22.71%
A:	36.64%	36.68%
G:	18.58%	18.54%

doi:10.1371/journal.pone.0143827.t004

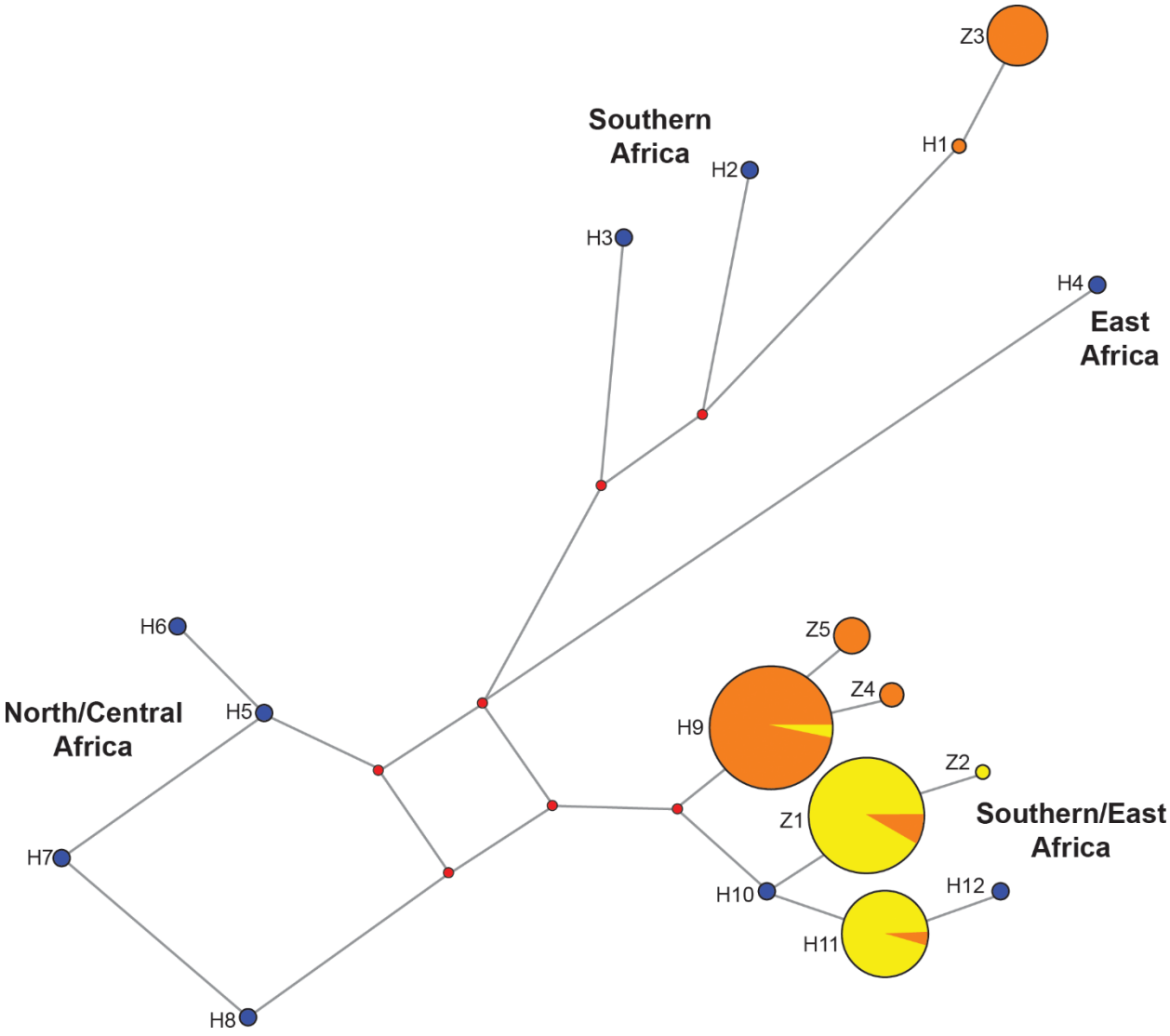


Figure 3.4. Median-joining network of 12S-16S haplotypes. Orange indicates haplotypes found in the western sub-population and yellow indicates haplotypes found in the eastern sub-population. Circle sizes of haplotypes found in Zambia are proportional to haplotype frequency. Red circles indicate median vectors. Blue circles indicate haplotypes not found in Zambia. H1-H12 are haplotypes which were described by Antunes *et al*.[38] and Z1-Z5 are novel haplotypes so far only found within Zambia. (Reprinted with permission from Curry *et al*. 2015)

DISCUSSION

Whether considered as a single population or two sub-populations, information from this study support the idea that Zambian lions represent a genetically diverse and healthy population.

Gene diversity, as defined here, represents the probability that any two sampled individuals within the population will have different haplotypes[116]. The overall gene diversity for Zambian lions sampled in this study was high (0.7319+/-0.0174). Even when considered as two sub-populations, gene diversity in Zambia's lions was higher than reported for lions in other regions (Kruger = 0.41, Namibia = 0.21, Northern Botswana = 0.29, and Serengeti = 0.03)[38]. Zambia's eastern and western sub-populations each showed similar gene diversity (approx. 0.5). The decrease in gene diversity from a single Zambian population to two regional sub-populations is due to some haplotypes occurring in only one of the two sub-populations and the presence of crossover haplotypes, which occur in both sub-populations (H9, H11 and Z1) but are common in one and rare in the other.

While gene diversity countrywide was high, matrilineal gene flow between regional sub-populations appeared to be low. F_{ST} between regional sub-populations was high at 0.47 (p-value<0.001) while F_{ST} calculated within a regional sub-population was 0.05. Values of F_{ST} greater than 0.25 suggest there is a high level of genetic differentiation between populations; a result of low gene flow[124]. Further evidence of differentiation at the sub-population scale is provided by Nei's distance (d) measures being highest between the eastern and western regions (Figure 3.3). Higher distance measures assume differences are caused more by mutation and genetic drift as opposed to migration, suggesting a low number of migrants between regions[125]. When considered as two regional sub-populations, the high F_{ST} and distance values between the eastern and western sub-populations coupled with low F_{ST} and distance values between areas within sub-populations suggests there to be little to no matrilineal gene flow between the eastern and western sub-populations while there is considerable movement within the eastern and western sub-populations.

Phylogenetic analysis is consistent with previous studies that postulate eastern-southern Africa as being the evolutionary cradle of the lion[30,39], supporting the hypothesis that Zambia may act as a genetic corridor between lion populations in eastern and southern Africa. The haplotypes present in the Zambian lion population were also found in both the Southern Africa lineage as well as the Southern/East Africa lineage described by Antunes et al.[38]. This grouping is parsimonious with studies that examined HVR1[39] and Cytochrome b[30] mtDNA sequences that grouped lions into two clusters, with Zambian lions falling within the Eastern and Southern Africa cluster. Cytochrome b analysis determined the Eastern and Southern Africa cluster to be more diverse than the North, West and Central cluster although the former cluster had weaker support[15]. Dubach et al.[15] also reported a lack of gene flow between most lion conservation units (LCUs) although microsatellite analysis indicated a high level of admixture in Botswana, Namibia and Zambia.

Analysis of mtDNA data indicates minimal gene flow between Zambia's two sub-populations; however, because it only establishes matrilineal distribution, whether the two sub-populations have historically experienced greater gene flow through higher levels of dispersal or if geographic separation has always inhibited lion movements between the eastern and western regions is unknown. Limited dispersal may still occur but not at a rate sufficient enough to maintain or increase the frequency of crossover haplotypes (i.e. H9 in LV and H11 in KF). All individuals with crossover haplotypes were male, a pattern consistent with a genetic population structure of high male dispersal and low female dispersal[113]. In African lions, males are more likely to disperse across farther distances[6,9,44,126] and are, therefore, more likely to cross geographic barriers but are unable to pass on mtDNA genes.

The most widely dispersed haplotype was H11 (Figure 3.2). Primarily an East Africa haplotype, it was the only haplotype observed in a population in southern Botswana and is also found in low frequency in Kruger National Park[38]. The wide range of the H11 haplotype could indicate that it is an ancestral haplotype and/or that, historically, there may have been some dispersal. Lion translocations could also be a contributor to the range of this haplotype as previous microsatellite analysis has shown evidence of translocation within LCUs[15].

With translocation becoming a well-practiced technique to prevent inbreeding within populations closed to dispersal or immigration[17], it must be determined whether there needs to be a focus on maintaining genetic diversity throughout the entire population or if there needs to be a more narrowed focus to prevent the loss of genetic diversity between sub-populations. In the example of Zambia, the question may be whether to prioritize maintaining genetic diversity throughout the country as a single population or if a more narrowed focus could serve to prevent the loss of genetic diversity between regional sub-populations. AMOVA analysis revealed little to no gene flow between the two sub-populations of lions within Zambia, a lack of genetic connectivity likely attributable to an expanse of cities and roads that inhibit modern day dispersal.

Further research including the addition of microsatellite analysis is being done to better quantify the level of overall genetic diversity within the population. The combination of mtDNA with nuclear markers will give a clearer picture to examine population-wide gene flow, identify evolutionarily distinct populations and calculate effective population size.

The findings of this study coincide with range-wide studies that propose lions are structured by region due to a lack of widespread movement of lions[15,38,127]. Existing regulatory measures aimed at improving lion conservation consider African lion at the species (P*anthera*

leo)[128,129] or subspecies (*Panthera leo ssp. leo*[130]) level, with the Asiatic lion always considered as a subspecies (*Panthera leo persica*[128,131]*, Panthera leo ssp. persica*[129]). Alternatively, some studies have considered management of lions at the sub-population level[7,15,16,38,40,41,45,46,127]. In West Africa, recommendations have been made to manage the small, isolated populations of lions as separate entities to allow for site-specific management and legislation[40,41]. The determination of regional sub-populations of lions in Zambia could be an important step for the creation of national wildlife management and legislation to preserve genetically healthy populations, ideally through the maintenance or restoration of natural connectivity at the landscape scale.

CHAPTER IV

GENETIC ANALYSIS OF AFRICAN LIONS IN ZAMBIA SUPPORT MOVEMENT ACROSS ANTHROPOGENIC AND GEOGRPHICAL BARRIERS*

INTRODUCTION

Zambia has one of the largest wild lion populations with a current estimate of around 1,200 individuals[132] within a range of more than 200,000 sq-km[133]. While lions do exist in small numbers in outlying areas of Zambia (e.g., Liuwa Plains National Park, Mweru Wantipa National Park), lion distribution can generally be divided into two regions (eastern and western) that are separated by geographic and anthropogenic features.

Zambia is one of nine countries with over 1,000 lions and has 2 of the 10 lion strongholds[19]. Kafue National Park (NP) and the adjoining Game Management Areas (GMAs) in the western part of the country collectively form the Greater Kafue Ecosystem (GKE). The GKE is designated a potential stronghold due to heavy poaching of the prey base[19], a significant concern as a decrease in prey lowers carnivore carrying capacity[134].

The Luangwa Valley Ecosystem (LVE) is a lion stronghold[19]. It is an offshoot of the Great Rift Valley system along the Luangwa River consisting of three NPs, the North Luangwa NP, South Luangwa NP, and Luambe NP, and their surrounding GMAs. The presence of suspected endemic subspecies has been used as evidence of the Luangwa Valley's geographic isolation. This includes Thornicroft's giraffe (*Giraffa camelopardalis thornicrofti*) found to be

* Reprinted with permission from "Genetic Analysis of African Lions in Zambia Support Movement Across Anthropogenic and Geographic Barriers" by Curry, C. J., White, P. A. & Derr, J. N. PLoS One 14(5): e0217179 (2019).

genetically distinct by mtDNA analyses[135]. However, more recent genetic studies using nuclear loci no longer consider them as their own subspecies and, although geographically separated by 500-km, now group them with a neighboring population of the Masai giraffe (*Giraffa camelopardalis tippelskirchi*) subspecies[136]. Other presumed endemic subspecies include the Cookson's wildebeest (*Connochaetes taurinus cooksoni*)[137], a subspecies of Blue wildebeest and the Crawshay's zebra (*Equus quagga crawshayi*), which has a striping pattern unique to Zambia[138].

Zambia is one of a handful of countries that actively engages in wild lion monitoring, conservation, and management[139]. Lions are a protected species under the Zambia Wildlife Act No. 12 of 1998 making it illegal to hunt, kill, capture or be in possession of a lion specimen without a permit[139]. Trophy hunting of lion is legally conducted in many of the GMAs. However, in Zambia as in many other countries, human-lion conflict is the greatest threat to lions outside of protected areas (PAs)[139]. Increased human activity on the edges of NPs and into GMAs severely limits the movement of carnivores[140,141]. Therefore, it is relevant to consider population connectivity when formulating lion conservation strategies.

Much of the area between the LVE and the GKE is comprised of an anthropogenic patchwork of towns and farms and is, therefore, considered uninhabitable by lions[19]. This is supported by mitochondrial DNA (mtDNA) which shows little to no matrilineal gene flow between the eastern and western sub-populations[91]. However, studies using minimal sampling from Zambia found an admixture pattern suggestive of male-mediated gene flow[106]. A larger study including both nuclear and mtDNA data was needed to capture a more accurate representation of lion movement and diversity in the region. This study used nuclear and mitochondrial markers from 409 lions across Zambia (Figure 4.1) to assess population structure

and the potential for movement of lions between the Luangwa Valley and the Greater Kafue ecosystems.

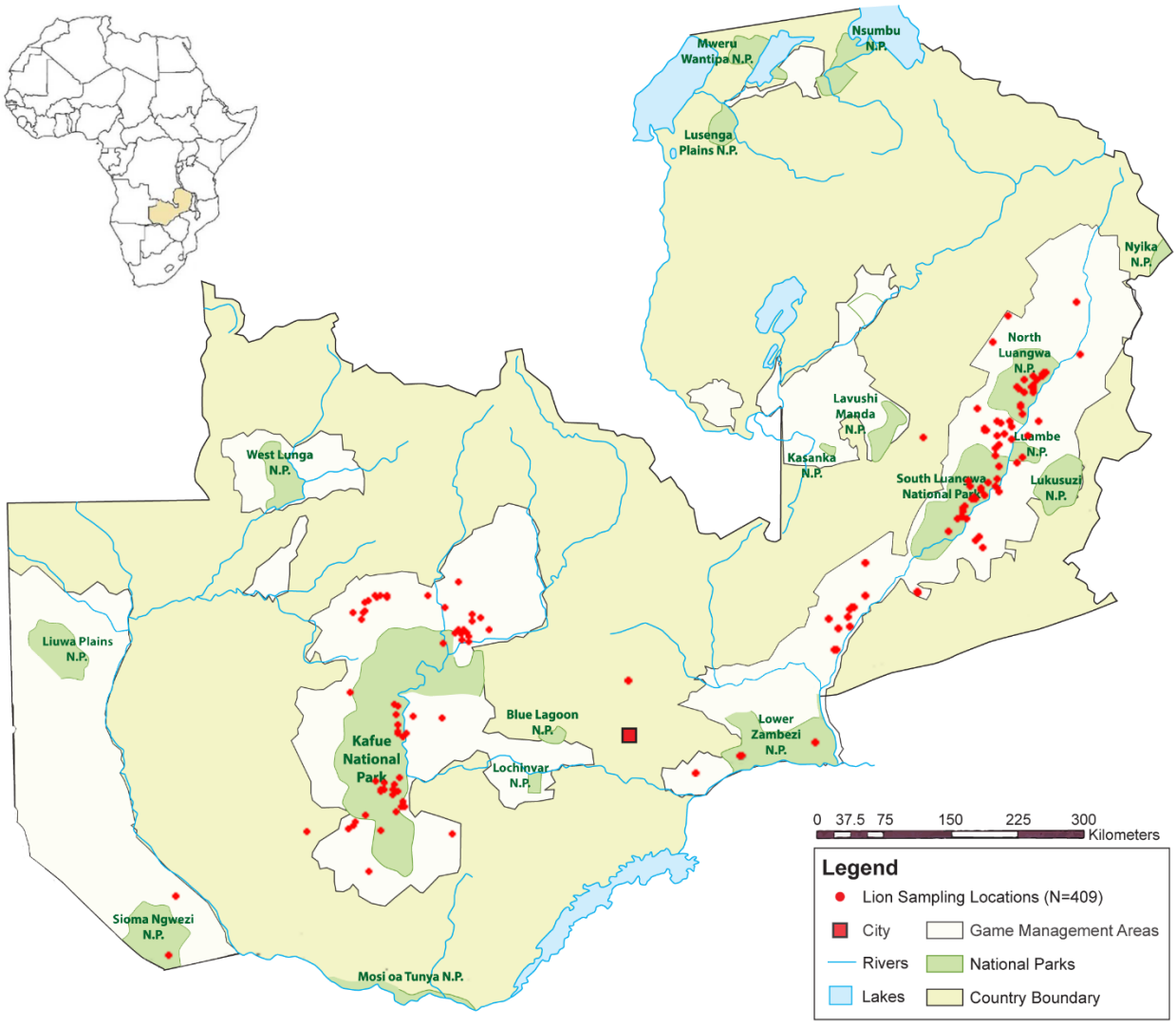


Figure 4.1. Map of Zambia with sampling locations. Sample locations are latitude and longitude coordinates of GPS location at sampling (181 lions at 181 locations) or a central location based on sampling area (228 lions at 33 locations). Detailed location information is in Appendix B.1.a. (Reprinted with permission from Curry *et al*. 2019)

MATERIALS AND METHODS

Sample Collection

The Zambia Lion Project (ZLP) provided 446 samples for analysis. Samples are in the form of hair, skin, bone and/or tissue and were collected during research conducted by ZLP in partnership with the Zambia Wildlife Authority (Research/Employment Permit No. #008872). For more details on sample collection, refer to Curry *et al*.[91].

Samples were collected between 2004–2012 in five of Zambia's National Parks (North Luangwa, South Luangwa, Lower Zambezi, Kafue and Sioma Ngwezi) and more than forty GMAs, making this dataset representative of Zambia's countrywide lion population. Each sample has an accompanying latitude and longitude sampling location. When exact sampling location could not be provided, a central latitude/longitude was given based on the sampling locale (see Appendix B.1.a).

DNA Extraction

DNA was successfully extracted from 421 samples of which 409 were further analyzed for mtDNA and microsatellites. Of the 12 samples eliminated, ten were duplicate individuals and two were not lions. The 409 samples analyzed consist of 324 males, 83 females, and 2 unknown (Appendix B.1.a), including all individuals previously studied in Curry *et al*.[91].

DNA was extracted using standard protocols and procedures used in the DNA Technologies Core Laboratory at Texas A&M University in College Station, TX (http://vetmed.tamu.edu/dnacore). PCR amplification was done using the KAPA Biosystems KAPA2G™ Robust HotStart PCR Kit with protocols described in Curry *et al*. [91] for mtDNA and *Curry & Derr* [142] for microsatellites. Cycling profiles for mtDNA and microsatellite

amplification can be found in S2 Appendix. PCR product was sequenced and/or genotyped on an Applied Biosystems 3130xl or 3730 Genetic Analyzer.

Mitochondrial DNA and Sequencing

Samples were amplified for a continuous region from the mtDNA 12S-16S genes using primers designed by Antunes *et al*.[38]. These primers were designed to prevent amplification of a 12.5 kb integration of mtDNA into the nuclear genome, or numt[56]. Sequences were manually edited and assigned a haplotype using SEQUENCHER v4.8[115].

Diversity calculations and phylogenetic analyses were carried out as in Curry *et al*.[91] so a direct comparison could be made with the larger sample size. The data was analyzed using Arlequin v3.5[116] as a full Zambian population and separated into eastern and western sub-populations. Number of polymorphic sites, gene diversity, nucleotide diversity and haplotype diversity were calculated for each. The sub-populations dataset was used for intra-population calculations of the coefficient of differentiation (F_{ST}) and hierarchical analyses of molecular variance (AMOVA). Finally, pairwise differences were calculated between sampling locations.

Phylogenetic analysis included all haplotypes from Antunes *et al*.[38] (GENBANK Accession #s FJ151641-FJ151652) and Zambian haplotypes found in Curry *et al*.[91] (GENBANK Accession #s KT164799-KT164803) and this study (ENA Accession #: LR593884). Phylogenetic analysis was performed using maximum likelihood analysis in PhyML 3.0[119] and Bayesian inference methods with Mr. Bayes[54,120]. For Bayesian analysis, samples were drawn every 1,000 steps over 50,000,000 MCMC steps with the first 10% discarded as burn-in and the tree was rooted with the tiger (*Panthera tigris;* GENBANK Accession #KJ508413) that was

aligned and trimmed to the lion sequences. A haplotype network was also made using the median-joining option of Network v4.6.1.3[123].

Microsatellites and Genotyping

A panel of 14 miniSTRs[142] (Leo006, Leo008, Leo031, Leo045, Leo077, Leo085, Leo098, Leo126, Leo224, Leo230, Leo247, Leo281, Leo391, and Leo506) were used for microsatellite analysis. Each sample was amplified, genotyped, and scored a minimum of two times at each locus to determine a consensus genotype. Samples were scored using STRand[143]. Samples scored at 10 or more loci were retained for further analysis.

Molecular diversity indices of expected and observed heterozygosity, deviation from Hardy-Weinberg equilibrium (HWE), conventional F-statistics, percentages of molecular variation, and number of migrants were calculated using Arlequin[116], GenAlEx[98], and GenePop[144]. Isolation-by-Distance (IBD) was calculated for females, males, and all individuals using the Mantel Test in GenAlEx[98]. Factorial Correspondence Analysis (FCA) and Principal Coordinate Analysis (PCoA) were carried out in GeneTix[145] and GenAlEx[98], respectively.

Number of alleles, number of private alleles and distribution of alleles were determined using GenAlEx[98] and allelic richness and private allelic richness was calculated using rarefaction in HPRare[146,147].

Effective Population Size was calculated using NeEstimator[148] using the linkage disequilibrium (LD) model. The two sub-populations have a high number of private alleles, many of which occur in low frequencies (<0.01). Because low allele frequencies can bias calculations of effective population size[149], the P_{crit} was set to frequencies of 0.00, 0.01, and to exclude only singleton alleles.

STRUCTURE[150] was used to evaluate population structure. Fifteen runs were performed for K=1 to 10 using 1,000,000 MCMC reps after 100,000 burn-in. Structure Harvester[151] was used to implement the Evanno method[152] to determine ΔK. CLUMPP[153] was then used to combine replicate runs and results were visualized using Distruct[154].

RESULTS

A total of 409 individuals were analyzed with 398 genotype panels and 391 sequences produced. Eighteen of the 398 genotyped individuals did not produce a haplotype. This is likely due to sample degradation. Degraded samples may not allow for amplification of large amplicons[155], such as this 1800-bp sequence of 12S-16S. Eleven individuals produced a haplotype but were not genotyped because DNA was lost in an accident after sequencing. These individuals didn't have more sample available for re-extraction of DNA.

Mitochondrial Diversity

A total of 391 samples produced full sequences (Appendix B.1.a). A novel haplotype, Z6, was found in three individuals in the eastern sub-population. Even with the addition of another haplotype, these results support the findings of Curry *et al.*[91]. Haplotype Z6 differs from haplotype H11 by one single nucleotide polymorphism (SNP) at position 1524 (Table 4.1). Distribution of haplotypes is shown in Table 4.2.

Table 4.1. Nucleotide position for each polymorphic site. (Reprinted with permission from Curry *et al*. 2019)

	242	369	393	462	513	531	571	596	631	684	695	732	798	812	840	841	928	929	961	1039	1082	1140	1220	1247	1328	1387	1524	1610	1629	1632	1646	1801
H1	C	C	A	T	C	G	G	A	T	T	G	C	T	G	T	A	A	A	A	C	C	A	A	T	A	T	T	C	C	T	C	A
H2	•	•	•	•	•	A	•	•	•	•	•	•	•	•	C	•	•	-	•	•	•	•	•	C	•	•	•	•	T	•	•	•
H3	•	•	•	•	T	A	•	•	•	•	•	•	•	•	•	•	•	-	G	•	•	•	G	•	•	•	•	•	T	•	•	•
H4	•	•	•	C	•	A	•	•	•	•	A	T	C	•	•	•	-	-	G	•	T	•	•	•	•	•	•	T	T	•	T	•
H5	•	T	•	•	•	A	•	•	C	•	•	•	•	•	•	•	-	-	G	•	•	•	•	•	•	•	•	•	T	•	T	•
H6	•	T	•	•	•	A	•	•	C	•	•	•	•	A	•	•	-	-	G	•	•	•	•	•	•	•	•	•	T	•	T	•
H7	T	T	•	•	•	A	•	•	C	•	•	•	•	•	•	•	-	-	G	•	•	G	•	•	•	•	•	•	T	•	T	•
H8	T	T	•	•	•	A	•	•	•	C	•	•	•	•	•	•	-	-	G	•	•	G	•	•	•	•	•	•	T	•	T	•
H9	•	•	•	•	•	A	•	•	•	C	•	•	•	•	•	G	-	-	G	•	•	•	•	•	G	•	•	•	T	•	T	•
H10	•	•	•	•	•	A	•	•	•	C	•	•	•	•	•	•	-	-	G	•	•	•	•	•	G	C	•	•	T	•	T	•
H11	•	•	•	•	•	A	•	•	•	C	•	•	•	•	•	•	-	-	G	T	•	•	•	•	G	C	•	•	T	•	T	•
H12	•	•	•	•	•	A	A	•	•	C	•	•	•	•	•	•	-	-	G	T	•	•	•	•	G	C	•	•	T	•	T	•
Z1	•	•	•	•	•	A	•	•	•	C	•	•	•	•	•	•	-	-	G	•	•	•	•	•	G	C	•	•	T	C	T	•
Z2	•	•	•	•	•	A	•	•	•	C	•	•	•	•	•	•	-	-	G	•	•	•	•	•	G	C	•	•	T	C	T	T
Z3	•	•	•	•	•	•	•	•	•	•	A	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•
Z4	•	•	G	•	•	A	•	•	•	C	•	•	•	•	•	G	-	-	G	•	•	•	•	•	G	•	•	•	T	•	T	•
Z5	•	•	•	•	•	A	•	G	•	C	•	•	•	•	•	G	-	-	G	•	•	•	•	•	G	•	•	•	T	•	T	•
Z6	•	•	•	•	•	A	•	•	•	C	•	•	•	•	•	•	-	-	G	T	•	•	•	•	G	C	C	•	T	•	T	•

*All nucleotide polymorphisms in 12S-16S are shown for haplotype H1. For all other haplotypes, only nucleotides that differ from H1 are shown.

**Haplotypes found in Zambia are in bold

Table 4.2. Distribution of Haplotypes. Number of males (♂), females (♀), and with unknown sex (?) for each haplotype is indicated along with the haplotype frequencies. (Reprinted with permission from Curry *et al*. 2019)

	Eastern			Western		Out				
Haplotype	♀	♂	**?**	♀	♂	♀	♂	n	***f***	**s.d.**
H1				0	1			1	0.003	0.003
H9	0	1	0	24	81	1	1	108	0.274	0.023
H11	15	59	1	0	2	0	1	78	0.202	0.020
Z1	23	125	1	0	3	1	0	153	0.391	0.025
Z2	0	1	0					1	0.003	0.003
Z3				10	27			37	0.095	0.015
Z4				0	4			4	0.010	0.005
Z5				5	1			6	0.015	0.006
Z6	0	3	0					3	0.008	0.004
Total	38	189	2	39	119	2	2	391		
	229			158		4				
A	5			7		3		9		

"Out" indicates samples collected outside PAs between sub-populations.
n = sample size; for ♂, ♀ and ? for each area and by area, haplotype and total
A = number of haplotypes
f = frequency
s.d. = standard deviation

AMOVA analysis, run as an eastern and western sub-population, resulted in an F_{ST} of 0.53 (p-value <0.001; Table 4.3). Gene diversity was calculated to be 0.7237 +/- 0.0112 for one Zambian population then 0.4712 +/- 0.0226 and 0.5041 +/- 0.0382 for the eastern and western sub-populations, respectively (Table 4.4).

Table 4.3. AMOVA results with F_{ST}. Percent variation is given among populations (Va) and within groups (Vb). The significance of differentiation within and among populations was tested by 1,000 permutations. (Reprinted with permission from Curry *et al*. 2019)

mtDNA

Source of Variation	d.f.	Sum of Squares	Variance Components	Percentage of Variation	p-value
Among Populations	1	232.299	1.237	53%	<0.001
Within Populations	385	419.076	1.089	47%	<0.001
Total	386	651.375	2.325		
Fixation Index	F_{ST}:	0.532			

STRs

Source of Variation	d.f.	Sum of Squares	Variance Components	Percentage of Variation	p-value
Among Populations	1	80.883	0.206	4%	<0.001
Within Populations	392	3720.861	4.801	96%	<0.001
Total	787	3801.745	5.007		
Fixation Index	F_{ST}:	0.041			

Table 4.4. Molecular diversity indices and nucleotide composition. (Reprinted with permission from Curry *et al*. 2019)

	Curry *et al.* 2015	This study
Nucleotide Sites	1882	1882
# Haplotypes	8	9
Polymorphic Sites	16	17
Transitions	13	14
Transversions	1	1
Indels	2	2
Composition		
C	22.11	22.12
T	22.67	22.66
A	36.64	36.65
G	18.58	18.57
Diversity		
Overall	0.7319 +/- 0.0174	0.7237 +/- 0.0112
Eastern	0.5057 +/- 0.0575	0.4712 +/- 0.0226
Western	0.5014 +/- 0.0336	0.5041 +/- 0.0382
F_{ST}	0.47	0.53

All trees resulted in similar clustering. The unrooted maximum likelihood tree is shown (Figure 4.2). The general configuration of the Bayesian phylogenetic tree and haplotype network did not change from Curry *et al.*[91], even with the addition of another novel haplotype. Haplotype Z6 appears as an additional branch to the H11 cluster, the second most predominant haplotype in the eastern sub-population.

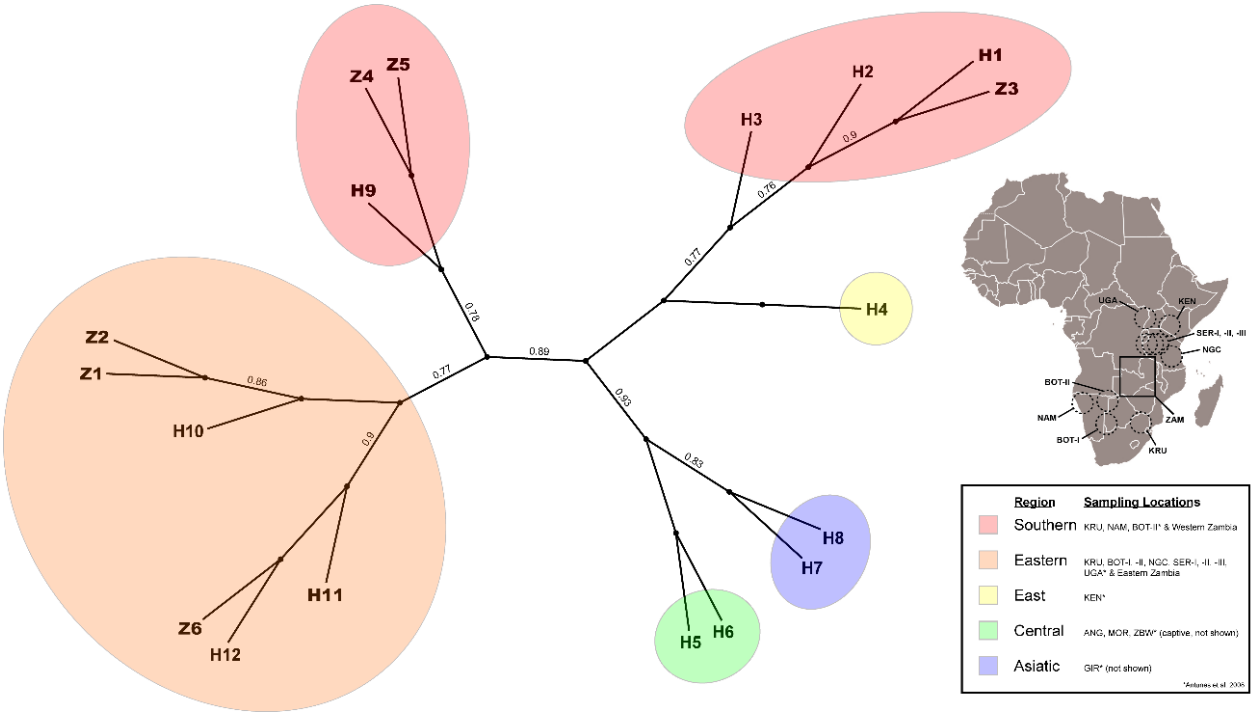


Figure 4.2. Radiated maximum likelihood tree with branch support. Clades are colored by region. Haplotypes in bold are found in Zambia. Map insert is from Curry *et al.*[91] showing locations of lions sampled. Circles indicate geographic locations for populations determined by Antunes *et al.*[38] UGA (Uganda); KEN (Kenya); SER (Serengeti NP); NGC (Ngorongoro Crater); KRU (Kruger NP); BOT-I (Southern Botswana and Kalahari); BOT-II (Northern Botswana); NAM (Namibia); GIR (India); ANG (Angola); ZBW (Zimbabwe); and MOR (Morocco). ZAM (Zambia) is denoted by a square. (Reprinted with permission from Curry *et al*. 2019)

Microsatellite Diversity

A total of 398 individuals were genotyped for 14 miniSTRs (Appendix B.1.a). Structure analysis revealed two sub-populations (ΔK=2; Figure 4.3) in agreement with mtDNA structuring[91]. Luangwa Valley (LV), Corridor (CO) and Lower Zambezi (ZA) of the LVE make up the eastern sub-population and Kafue (KF) and Sioma Ngwezi (SI) of the GKE make up the western sub-population. Little admixture is evident between sub-populations. Two individuals are assigned to a sub-population contrary to the location they were sampled. Individual 2011000432 sampled in KF is assigned to the eastern sub-population (0.81) and 2011000878 from north of North Luangwa NP is almost equally assigned to the eastern (0.43) and the western (0.57) sub-populations.

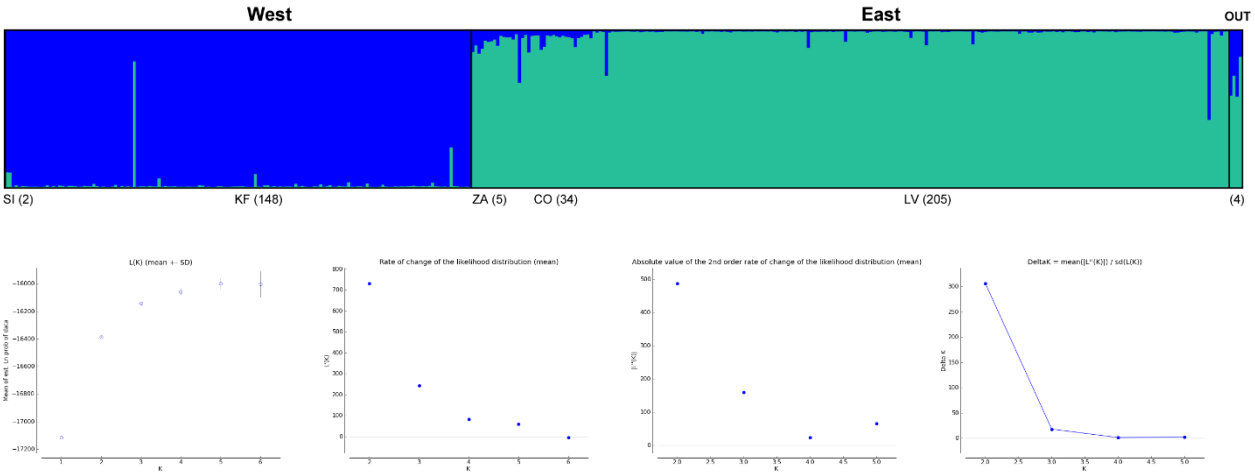


Figure 4.3. Results of STRUCTURE analysis based on 14 microsatellite loci of 398 Zambian lions. Population assignment results for ΔK=2 showing a separation of the western and eastern lion populations in Zambia supported by Structure Harvester plots. (Reprinted with permission from Curry *et al*. 2019)

Gene diversity, as established by expected heterozygosity (H_E), is high (Table 4.5) locus by locus and as the mean across loci for the population (0.701) and within sub-populations (eastern = 0.682 and western = 0.692). Significant deviations for HWE are seen in 13 of 14 loci when the population is considered as a whole. Separated into sub-populations, the eastern sub-population deviates from HWE at 4 loci (Leo126, Leo224, Leo230, and Leo 391) while the western sub-population deviates from HWE at only 3 loci (Leo230, Leo247, Leo247). The only shared loci that deviates from HWE is Leo230. For most loci that deviate from HWE, a deficiency of heterozygotes is observed (Table 4.5).

Number of alleles (A) and allelic richness (AR) are high across loci (Table 4.5). The number of private alleles (PA) is high for both sub-populations. There are 213 individuals with 1-4 PA at 1-3 loci (Table 4.6). For the eastern sub-population, there are 31 PA spanning all 14 loci with 144 of 244 individuals having at least one PA. The western sub-population has 14 PA in nine of 14 loci and 69 out of 150 individuals have at least one PA.

Weak but significant genetic structure is detected with an $F_{ST} = 0.04$ (p-value = 0.001; Table 4.3) attributing 4% of molecular variance among populations and 96% of molecular variance within populations. There is little evidence of inbreeding with an F_{IS} below zero (-0.034). The number of migrants per generation (Nm) was calculated to be 5.6 using two methods (GenAlEx and GenePop).

Table 4.5. STR Diversity Indices. (Reprinted with permission from Curry *et al*. 2019)

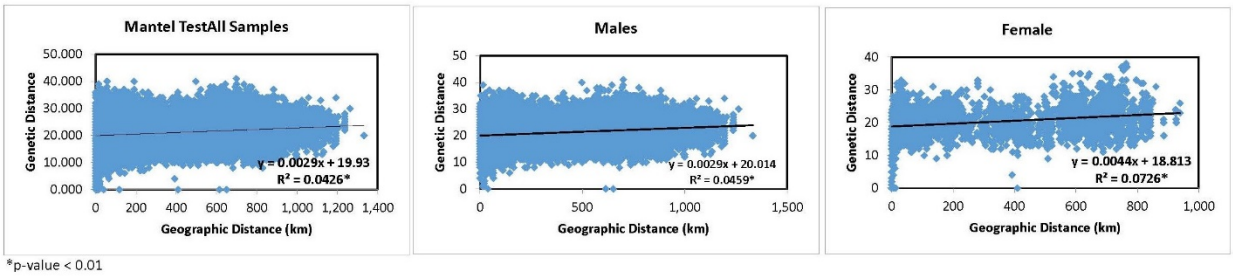
Locus	N	A	AR	PA	PAR	H_O	H_E	p-value	Signif
Leo006	397	14	13.85			0.763	0.835	0.000	***
East	*243*	*12*	*10.69*	*3*	*2.71*	*0.774*	*0.773*	*0.054*	*ns*
West	*150*	*11*	*10.70*	*2*	*2.72*	*0.747*	*0.810*	*0.545*	*ns*
Leo008	398	8	7.96			0.729	0.762	0.003	***
East	*244*	*7*	*6.78*	*1*	*0.92*	*0.705*	*0.694*	*0.504*	*ns*
West	*150*	*7*	*6.84*	*1*	*0.98*	*0.760*	*0.738*	*0.640*	*ns*
Leo031	396	7	6.74			0.407	0.387	0.025	*
East	*244*	*7*	*6.06*	*4*	*3.06*	*0.377*	*0.364*	*0.072*	*ns*
West	*148*	*3*	*3.00*	*0*	*0.00*	*0.446*	*0.407*	*0.396*	*ns*
Leo045	396	8	7.72			0.356	0.410	0.005	**
East	*242*	*7*	*6.07*	*3*	*2.08*	*0.455*	*0.503*	*0.163*	*ns*
West	*150*	*5*	*4.96*	*1*	*0.98*	*0.193*	*0.226*	*0.059*	*ns*
Leo077	398	7	7.00			0.746	0.740	0.040	*
East	*244*	*7*	*7.00*	*1*	*1.00*	*0.758*	*0.748*	*0.102*	*ns*
West	*150*	*6*	*6.00*	*0*	*0.00*	*0.733*	*0.716*	*0.355*	*ns*
Leo085	398	9	8.86			0.668	0.654	0.192	ns
East	*244*	*6*	*5.87*	*2*	*1.87*	*0.635*	*0.618*	*0.093*	*ns*
West	*150*	*7*	*6.86*	*3*	*2.86*	*0.720*	*0.686*	*0.628*	*ns*
Leo098	398	8	7.86			0.661	0.702	0.013	*
East	*244*	*8*	*7.46*	*2*	*1.51*	*0.623*	*0.671*	*0.137*	*ns*
West	*150*	*6*	*6.00*	*0*	*0.05*	*0.727*	*0.706*	*0.088*	*ns*
Leo126	397	10	9.86			0.688	0.758	0.000	***
East	*243*	*10*	*9.43*	*3*	*1.85*	*0.658*	*0.744*	*0.000*	***
West	*150*	*8*	*7.58*	*0*	*0.00*	*0.740*	*0.757*	*0.335*	*ns*
Leo224	398	9	8.98			0.668	0.711	0.000	***
East	*244*	*8*	*7.75*	*3*	*2.05*	*0.660*	*0.711*	*0.000*	***
West	*150*	*7*	*6.72*	*1*	*1.02*	*0.673*	*0.701*	*0.132*	*ns*
Leo230	344	12	12.00			0.738	0.812	0.000	***
East	*211*	*10*	*9.55*	*2*	*1.55*	*0.701*	*0.795*	*0.000*	***
West	*129*	*10*	*10.00*	*2*	*2.00*	*0.798*	*0.825*	*0.000*	***
Leo247	398	9	8.96			0.759	0.806	0.000	***
East	*244*	*8*	*7.78*	*1*	*0.78*	*0.791*	*0.813*	*0.063*	*ns*
West	*150*	*8*	*7.98*	*1*	*0.98*	*0.713*	*0.787*	*0.001*	***
Leo281	398	16	15.85			0.646	0.693	0.000	***
East	*244*	*15*	*13.83*	*2*	*1.72*	*0.635*	*0.634*	*0.289*	*ns*
West	*150*	*14*	*13.57*	*1*	*1.46*	*0.667*	*0.758*	*0.000*	***
Leo391	397	9	8.98			0.695	0.740	0.040	*
East	*244*	*9*	*8.78*	*2*	*1.78*	*0.652*	*0.695*	*0.002*	**
West	*149*	*7*	*7.00*	*0*	*0.00*	*0.765*	*0.778*	*0.924*	*ns*
Leo506	391	11	10.86			0.785	0.807	0.018	*
East	*242*	*9*	*8.10*	*2*	*2.40*	*0.777*	*0.793*	*0.082*	*ns*
West	*145*	*8*	*7.89*	*2*	*2.19*	*0.800*	*0.789*	*0.208*	*ns*
Mean	***393***	***9.79***	***9.68***			***0.665***	***0.701***	***0.024***	*****
East	*241*	*8.79*	*8.22*	*2.21*	*1.81*	*0.657*	*0.682*	*0.112*	*ns*
West	*148*	*7.64*	*7.51*	*1.00*	*1.09*	*0.677*	*0.692*	*0.308*	*ns*

N, Sample Size; A, Number of Alleles; AR, Allelic Richness; PA, Number of Private Alleles; PAR, Private Allelic Richness; H_O, Observed Heterozygosity; H_E, Expected Heterozygosity; p-value for deviation from HWE
ns, not significant; * p-value < 0.05; ** p-value < 0.01; *** p-value < 0.001

Table 4.6. Number of individuals with private alleles (PA). $N_{\#PA@\#Loci}$ is the number of private alleles at the given number of loci. Totals are given for the total number of individuals with any PA (N_{wPA}), number of individuals homozygous for a PA (N_{Hom4PA}), i.e. two of the same PA at the same locus for one or more loci, and number of individuals heterozygous for a PA (N_{Hom4PA}), i.e. two different PA at the same locus for one or more loci. (Reprinted with permission from Curry *et al*. 2019)

	Eastern	Western	Total
N_{wPA}	**144**	**69**	**213**
$N_{wPA@1Locus}$	*86*	*46*	*132*
$N_{wPA@2Loci}$	*44*	*23*	*67*
$N_{wPA@3Loci}$	*14*	*0*	*14*
$N_{wPA@>3Loci}$	*0*	*0*	*0*
N_{Hom4PA}	**10**	**2**	**12**
$N_{2PA@1Locus}$	*3*	*2*	*5*
$N_{3PA@2Loci}$	*5*	*0*	*5*
$N_{4PA@3Loci}$	*2*	*0*	*2*
N_{Het4PA}	**6**	**1**	**7**
$N_{2PA@1Locus}$	*3*	*1*	*4*
$N_{3PA@2Loci}$	*2*	*0*	*2*
$N_{4PA@3Loci}$	*1*	*0*	*1*

A Mantel test was done for males and females separately to account for possible dispersal differences between sexes due to their mating system [6,9,126]. Both males and females show a low but significant level of IBD (Male Rxy=0.214, p-value=0.01; Female Rxy=0.269, p-value=0.01; Figure 4.4). Factorial Correspondence Analysis (FCA; Figure 4.5) and Principal Coordinate Analysis (PCoA; Figure 4.6) reveal that LV and KF cluster separately with CO and ZA as intermediaries, overlapping with both LV and KF. Finally, Nei's distances are higher between western and eastern regions (Figure 4.7).



*p-value < 0.01

Figure 4.4. Genetic distance versus geographic distance Mantel Tests.

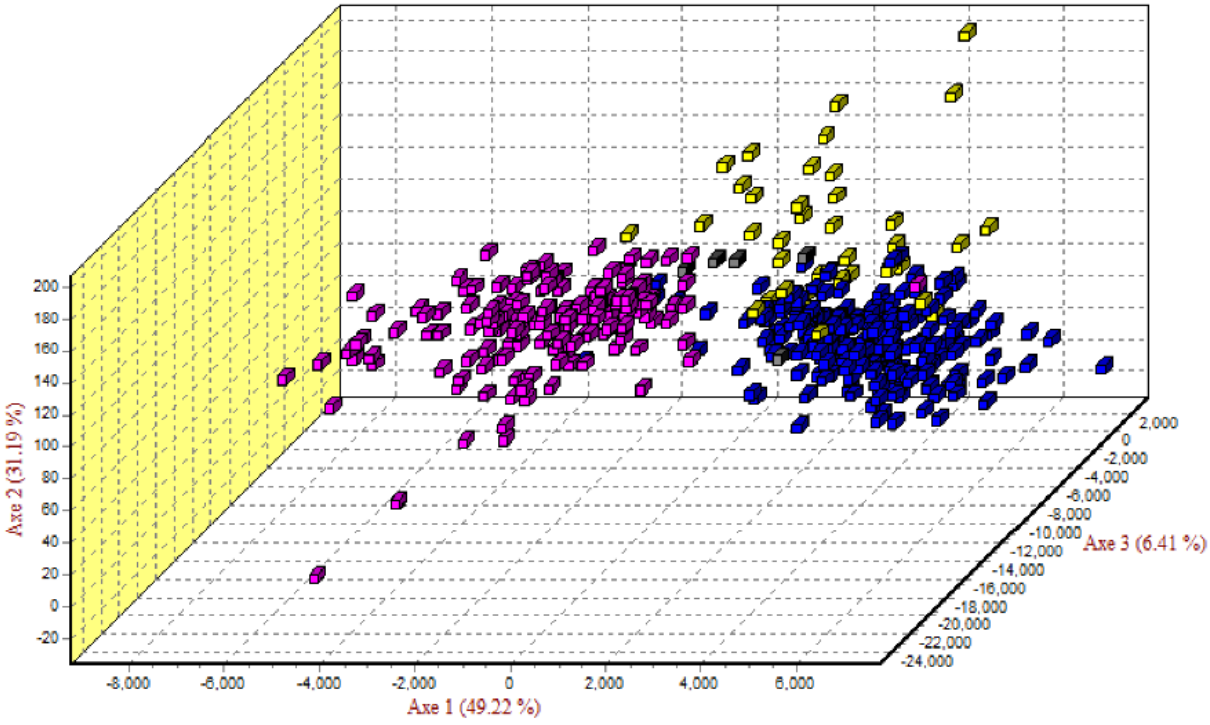


Figure 4.5. Factorial correspondence analysis (FCA) based on 14 microsatellite loci of 398 Zambian lions. Pink = Western Zambia (KF & SI), Blue = Luangwa Valley (LV), Yellow = Lower Zambezi NP and eastern corridor (ZA & CO), Grey = Outside PAs. Axe 1, 2, and 3 represent 86.82% of the genetic variation observed. (Reprinted with permission from Curry *et al*. 2019)

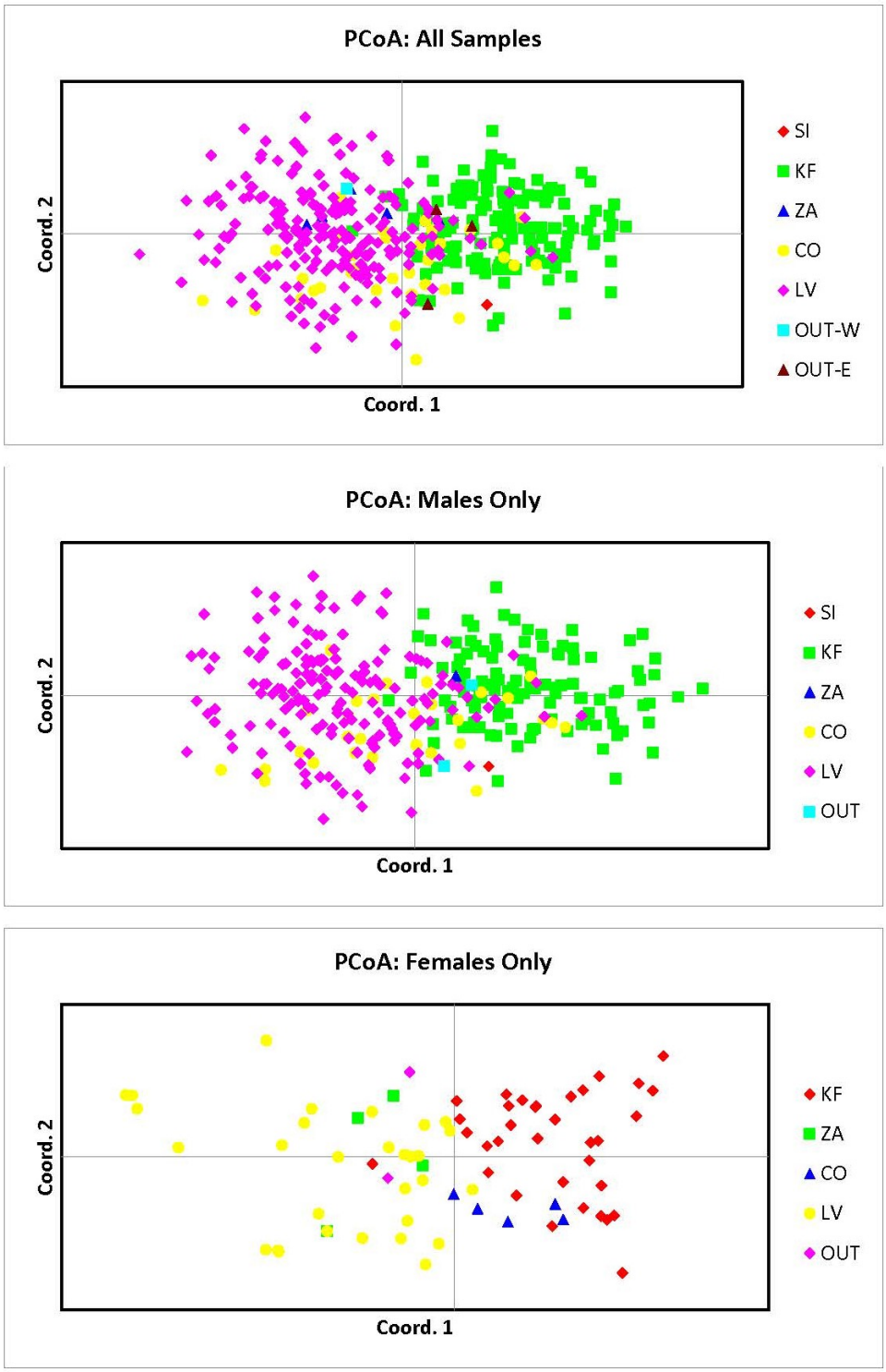


Figure 4.6. Principal coordinate analysis (PCoA) of genetic distance.

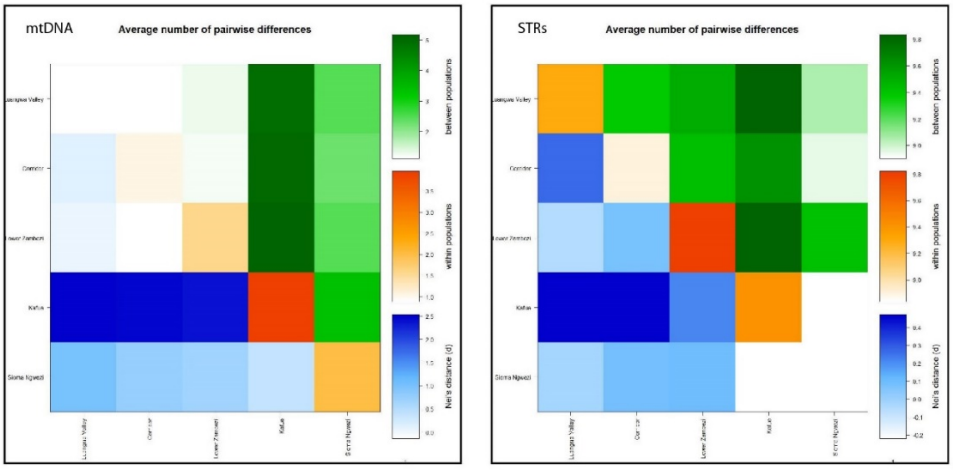


Figure 4.7. Average number of pairwise differences between populations (above diagonal), within populations (diagonal), and Nei's corrected (below) for mtDNA and STRs.

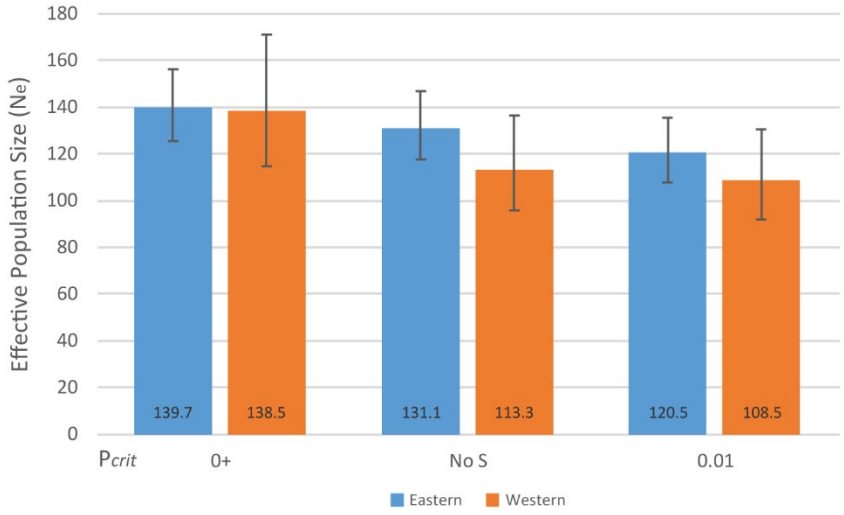


Figure 4.8. Effective population size (N_e) calculations with upper 95% confidence intervals. Estimates of N_e with P_{crit} set to frequencies of 0.00 (0+), 0.01, and to exclude only singleton alleles (No S). (Reprinted with permission from Curry *et al*. 2019)

Effective population size (N_e) calculations with upper 95% confidence intervals are shown in Figure 4.8 for the eastern and western sub-populations.

DISCUSSION

Increasing the sample size did not change the gene diversity of lions within the western sub-population of Zambia, nor did it change gene diversity at the country-wide scale[91]. Gene diversity of the eastern sub-population was lowered slightly, but still remains high (Table 4.4). The addition of microsatellite analysis supports earlier findings that Zambia's lion population is highly diverse (Tables 4.5 and 4.6). Furthermore, with an F_{IS} value close to zero, Zambian lions do not show any detectable evidence of inbreeding.

The addition of another novel haplotype (Z6) did not change the configuration of the phylogenetic tree or haplotype network. Haplotype Z6 is one SNP from H11, clustering with H11 and H12 within the East/Southern Africa cluster. H11 is the most wide-spread haplotype, found in individuals as far north as Uganda and south as Kruger NP[38]. Most Zambian haplotypes cluster together within two branches of the East/Southern Africa cluster. Haplotype Z3, found only in Kafue NP, is in a different cluster with other southern haplotypes. This clustering suggests connectivity of the western sub-population southwest while the eastern sub-population has connectivity both north and south but remains to the east.

Mitochondrial analysis shows minimal gene flow between populations ($F_{ST} = 0.53$) while microsatellite analysis suggests greater gene flow ($F_{ST} = 0.04$). F_{ST} for microsatellites is the measure of the heterozygote deficit relative to its expectation under HWE[156] while F_{ST} for mtDNA is a function of the number of mutations between molecular haplotypes as measured by haploid diversity[116]. These differences in concept could also be a reason for the vast differences between the two F_{ST} values. However, this pattern could also be a result of the mating system where males disperse across farther distances than females[9]. Males are more likely to disperse, passing along their nDNA but unable to pass on mtDNA genes.

Pairwise differences (Figure 4.7) for nDNA and mtDNA also show evidence of the mating system. Nei's distances are higher between western and eastern regions. For nDNA, however, distances are lower between ZA in the east and KF in the west, indicating this is likely the region genetic movement between the eastern and western sub-populations occur. Further evidence of this movement is shown in FCA and PCoA analyses (Figures 4.5 and 4.6).

Migration is the transfer of genetic variation from one population to another[157]. Mutation cannot be disentangled from differences introduced by migration so F_{ST} can underestimate differentiation in a highly structured population[156], particularly when other metrics support strong structure. Migration is evident within Zambia ($Nm = 5.6$), therefore, F_{ST} calculated for microsatellites, could be an underestimation based on high migration coupled with the distinct structure shown in other analyses. However, as migration increases, the proportion of private alleles will decrease. If gene flow is low, there are more private alleles, and if gene flow is high, private alleles are more rare[157]. Zambia has a high number of private alleles with 31 private alleles in the eastern sub-population and 13 in the western sub-population found in 213 individuals. A majority of these alleles are in low frequency, however, 13 appear in frequencies greater than 1% of the sampled population.

Structure analysis shows two distinct sub-populations with admixture present in only a few individuals (Figure 4.3). 2011000878, sampled in a GMA north of North Luangwa NP, is the most admixed individual, assigned almost equally to each sub-population, implying it is the offspring of a resident and migrant mating. A possible migrant, 2011000432, was sampled in the western sub-population but is assigned to the eastern sub-population. This same individual was previously flagged as a possible migrant based on its mtDNA haplotype[91].

Further support for substructure is the presence of a Wahlund effect. This is when the subdivision of genetically distinct demes causes a deviation from HWE at the population level resulting in the appearance of a deficit of heterozygotes[158–161]. Across Zambia, mean H_O versus H_E shows a heterozygote deficiency at the population level, however, when separated into sub-populations, mean values no longer deviate from HWE (Table 4.5). This same pattern is present across loci with a deviation from HWE at the population level and being in HWE at sub-population level. Leo230 is the only locus that remains out of HWE at the sub-population level. This may be a result of issues with the locus, as this was the only locus that exhibited problems with amplification.

With evidence of clusters, substructure, and Wahlund effects, it is suggested to remove migrants from the population before estimating N_e[162]. Therefore, migrants and individuals found outside PAs were removed before calculating N_e. The LD method was used because it is robust and mostly unbiased at a sub-population level[163]. In simulations of single-sample N_e estimators, the LD method performed best producing estimations of N_e closest to the true value of N_e[163–165]. Heterozygote excess and molecular co-ancestry methods often have poor precision in comparison[162,165].

The population size of lions in Zambia during the sampling period was estimated to be as low as 700 lions with 250-500 in Kafue NP, 400-750 in the Luangwa Valley, and <50 in the corridor and Lower Zambezi NP[2]. N_e for the eastern and western sub-populations is calculated to be between 100-200, a lower value than what would be expected for a large and diverse lion population[11]. Lion prides typically have multiple related females mating with 1-7 males that originated from a different pride or prides[9]. This type of polygynous mating system can lower the value of N_e depending on the number of males breeding within each pride[11].

When loci are physically unlinked, LD is caused by drift, migration, or selection. Assuming neutral loci in an isolated population with random mating, LD would be a result of drift alone and can be used to calculate N_e[166]. However, LD calculated from a sample from a sub-population can lead to an underestimation of local N_e when the migration rate is low[163,165]. The number of migrants between the eastern and western sub-populations is calculated to be less than six individuals per generation. This is a migration rate well below 5-10%[163]. Therefore, this could be an underestimation of N_e (Figure 4.8) as a result of migration between sub-populations.

Overall, Zambia has a genetically diverse population of lions, although effective population size appears to be lower than expected. Previously thought to be isolated via anthropogenic and geographic barriers, the eastern and western sub-populations do exhibit isolation-by-distance, with a low level of migrants per generation. This migration may cause an underestimation of effective population size but is maintaining and introducing diversity across Zambia.

Translocation is a well-practiced technique to prevent inbreeding[17]. Zambia does not appear to be in need of using translocation as a management strategy. While maintaining genetic diversity throughout the entire population should be considered, the high number of private alleles present within each sub-population and the level of population substructure found suggests there should be a more narrowed focus to prevent the loss of genetic diversity within sub-populations. Maintenance of diversity across Zambia will still occur through gene flow of lions between sub-populations, as it has been occurring already without intervention. This is assuming that future connectivity between sub-populations stays the same, or improves, rather than decreasing.

Range-wide studies have proposed lions are mostly structured by region due to restricted widespread movement of lions across the landscape[15,38,127]. Findings in this study agree, though movement outside protected areas is occurring. To further augment gene flow, historic or present-day corridors would need to be created and protected to help ensure continued natural dispersal of lions between the eastern and western sub-populations.

CHAPTER V

GENETIC DIVERSITY OF THE LION ACROSS SPACE & TIME

INTRODUCTION

The lion population has changed dramatically over the past 100 years, particularly in response to habitat availability and effects of a growing human population[19]. Around the turn of the 19th century, explorers, naturalists and hunters went on expeditions to collect biological specimens for preservation in natural history museums. These expeditions resulted in hundreds of lion specimens preserved in museums across the world that predate the precipitous human population growth across Africa[167,168]. With the development of techniques and technologies for improved isolation and sequencing of degraded genetic material (aDNA), these collections give us access to genetic information from long dead individuals as well as their contemporary counterparts.

Multiple published investigations document the genetic consequences of large scale landscape changes over a short amount of time[169–172] (i.e. 100 years). Habitat fragmentation can change allelic and haplotype frequencies and distribution[173]. Levels of genetic diversity are directly proportional to a species ability to adapt, survive, and thrive and loss of genetic diversity is detrimental to overall population health and long-term survival. The adverse effects of low genetic diversity have been observed in small populations of African lions that exist in heavily managed fenced reserves[17]. It is difficult, however, to predict how losses in the genetic diversity within the wild lion population will negatively impact its overall health.

Studies with historical and ancient lions have been primarily restricted to mtDNA analyses incorporated within a modern lion dataset [30,31,33,39,55]. A recent study including

historical-aged individuals focused on assessing changes in the recent past but was confined to a local analysis of the Kavango–Zambezi transfrontier conservation area (KAZA)[49]. By separating out the historical-aged individuals from recent modern individuals in a range-wide study, we can identify the extent of change in genetic diversity that has occurred in this time of landscape and anthropogenic change[83,174].

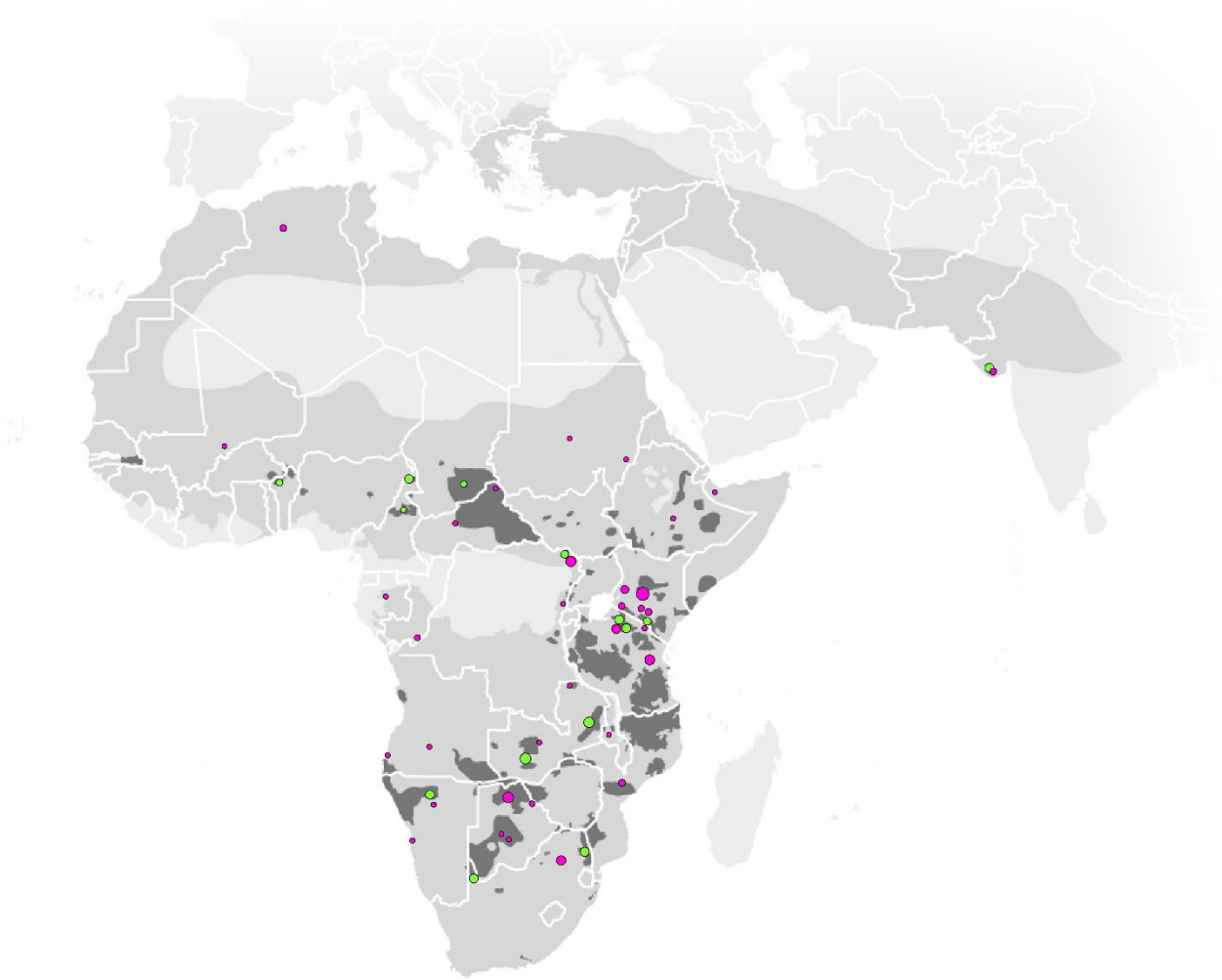


Figure 5.1. Map of Lions Sampled. Green dots are modern sample locations. Pink dots are historical sample locations. Dot size coincides with sample size for each location. Medium gray is the historical range. Dark grey is the current range.

METHODS

Historical Lion DNA

Biological material from 151 lions dating prior to 1947 was collected from museums (Figure 5.1, Table 5.1) in the form of bone fragments, whole teeth or tooth fragments, nasal turbinate bones, and/or dried tissue. Detailed protocols of sample preparation, DNA extraction, and storage can be found in Appendix A. DNA from 11 additional historical samples was provided by the Institute of Environmental Sciences Leiden.

Table 5.1. List of natural history museums that provided lion samples for the historical population.

Museum	*Location*	Provided	nDNA	mtDNA
American Museum of Natural History (AMNH)	*New York, NY, USA*	96	80	71
Carnegie Museum of Natural History (CM)	*Pittsburgh, PA, USA*	11	4	4
Field Museum of Natural History (FMNH)	*Chicago, IL, USA*	30	30	18
Kansas University Natural History Museum (KU)	*Lawrence, KS, USA*	2	2	2
Natural History Museum of Los Angeles County (LACM)	*Los Angeles, CA, USA*	3	3	1
Naturalis Biodiversity Center (RMNH)	*Leiden, Netherlands*	4	4	0
Royal Belgian Institute of Natural Sciences (KBIN)	*Bruxelles, Belgium*	2	2	0
Swedish Royal Museum of Natural History (S)	*Stockholm, Sweden*	3	3	0
The Museum of Vertebrate Zoology at Berkeley (MVZ)	*Berkeley, CA, USA*	1	1	1
Yale Peabody Museum (YPM)	*New Haven, CT, USA*	8	6	5
Zoological Museum Amsterdam (ZMA)	*Amsterdam, Netherlands*	2	2	0

nDNA

Modern Lion Sample Selection

The modern dataset (MD) consists of microsatellite allele calls from Bertola *et al*. 2015[41] (MD-1), Discoll *et al.* 2002[73] (MD-2) and Curry *et al.* 2019[104] (MD-3). Six additional lions

amplified at all 14 Leo STRs[142] were included from the African Wildlife Genomics collection at Texas A&M University (MD-4). These datasets were combined to expand sample size and range for structure analysis and population statistics and direct comparison with the historical nuclear dataset (HD).

There is sampling overlap between MD-1, MD-2, and MD-3. Lions in MD-2 were included in MD-1[41], though MD-2 has more loci than MD-1. Nine lions from the eastern subpopulation in Zambia were used in both MD-1 and MD-3. MD-3 includes an additional 8 lions from the Zambian eastern subpopulation and 17 lions from the Zambian western subpopulations chosen at random to represent the known subpopulations in Zambia[104] at comparable sample sizes to subpopulations sampled in MD-1 and MD-2.

Data calibration is needed when combining microsatellite allele calls from different studies[175]. Leo STRs were necessary for increased amplification of the historical samples[142], therefore, MD-1 data were calibrated to MD-3. Two samples from MD-1 (*M_CAM_0019* and *M_BEN_0013*) were amplified and genotyped using FCA microsatellite primers and Leo STR primers for loci 006, 008, 031, 045, 077, 085, 098, 126, 224, 230, 247, 281, 391, and 506. The FCA, Leo, and published allele calls were compared to determine a correction for calibration of the allele calls for the two studies (Appendix C3). This correction was then applied to all other allele calls for the appropriate loci. The 9 lions that appear in both MD-1 and MD-3 provide a secondary check for the accuracy of the calibration.

Historical Lion STR Amplification

The HD consists of the 162 museum collected lions. Microsatellite amplification was done following protocols and procedures described in Curry and Derr 2019[142] for all 14 Leo

STRs. Detailed protocols for PCR amplification, allele calling, and call verification can be found in Chapter 2 and Appendix A.

Analyses

Nine loci (Leo006, Leo008, Leo085, Leo098, Leo126, Leo224, Leo230, Leo247, Leo281) had greater than 75% coverage across both MD and HD. These loci were used for further analysis. Diversity calculations were done using Arlequin v3.5[116], GenePop[144], HPRare[146], and GenAlEx v6.5[98]. All analyses were done to the MD and HD separately then compared. A comparison of means was used to determine statistical significance of differences between historical to modern metrics.

Knowing that structure population has been found regionally[31,38,48,75,76,176,177], we implemented a hierarchical strategy to uncover any hidden structure that may be lost when subpopulations are sampled together[178–180]. The full dataset was run without priors for 15 iterations of K 1-15 for 100,000 MCMC reps with 10% burn in. STRUCTURE was rerun for each population as determined through ΔK values from STRUCTURE HARVESTER[151] with individuals assigned to populations based on Q scores from runs combined in CLUMPP[153]. To determine structural tiers, this was continued until no additional population structure was found. Samples were assigned to the finest level of structure then run as a full population with location priors for 15 iterations of K 1-12. Runs were combined using CLUMPP[153] and visualized at each tier using DISTRUCT[154]. To further look at structure patterns, a mantel test for isolation-by-distance (IBD) and principal coordinate analysis (PCoA) was done in GenAlEx v6.5[98].

Samples were grouped in conventionally recognized regions Northern, Western, Eastern, and Southern (Figure 5.2) based on sampling location. A phenetic tree based on D_A genetic

distance between sampling groups within datasets was constructed in POPTREE2[181] using the neighbor-joining method with 1,000 bootstraps performed.

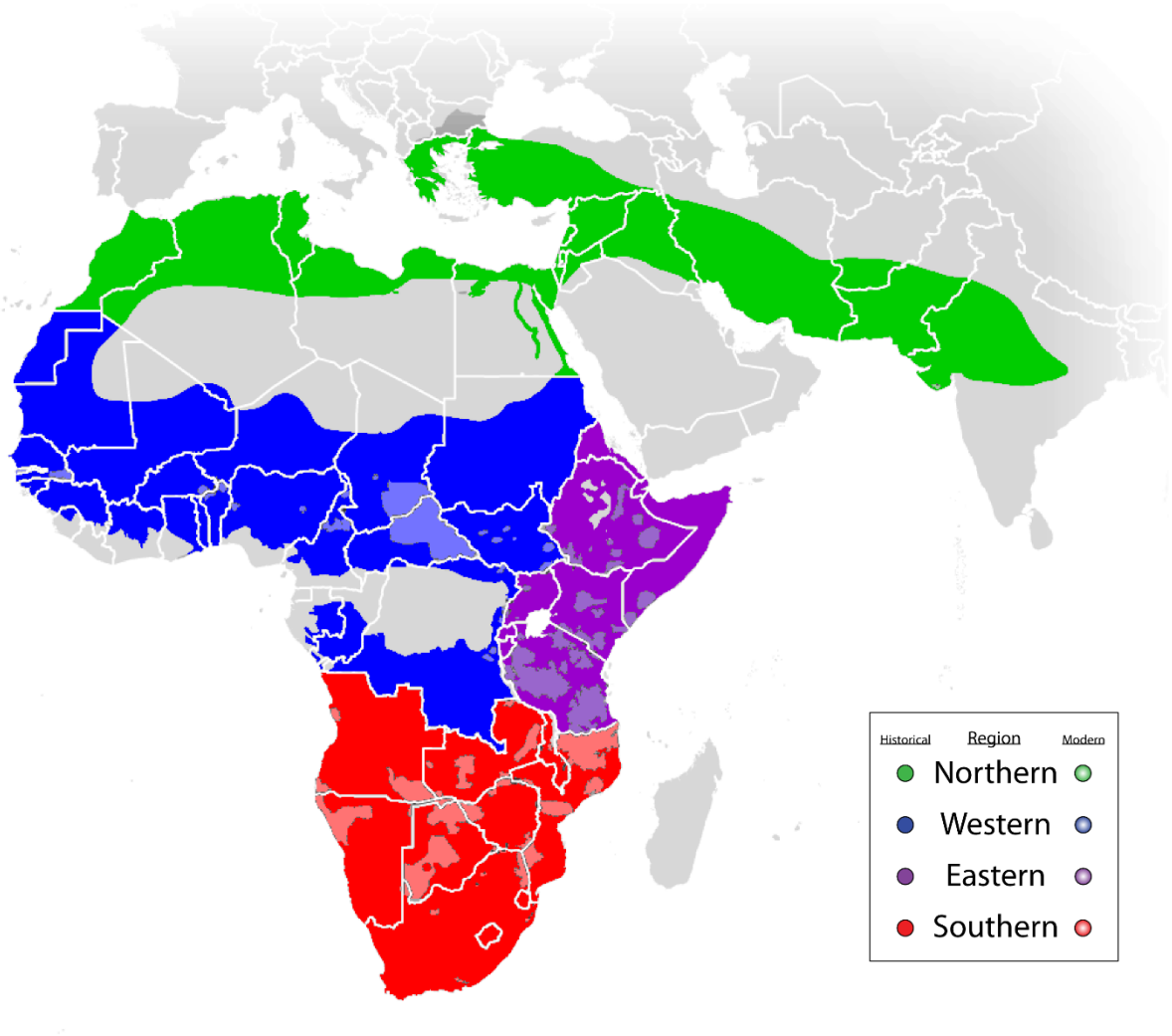


Figure 5.2. Map denoting sample groups. Groups are based on conventionally recognized regions and subcontinental population structure.

mtDNA

Whole Genome Sequencing

To obtain mitogenomes, 155 samples were whole genome sequenced. Library prep and sequencing were performed by Texas A&M AgriLife Genomics and Bioinformatics Services. Library prep was done using NEBNext® Ultra™ II, designed for low input amounts, and sample libraries were sequenced on the Illumina HiSeq 4000. Sequence cluster identification, quality prefiltering, base calling, and uncertainty assessment were done in real time using Illumina's HCS 2.2.68 and RTA 1.18.66.3 software with default parameter settings.

Reads were mapped by SpeedSeq[182] to the lion genome reference (provided by Ellie Armstrong at Stanford University) with a mitogenome sequence (GenBank Accession KP001505.1) and representative nuclear mitochondrial sequence (personal communication, Dr. Laura Bertola) added as additional scaffolds. The *Panthera* lineage contains multiple nuclear mitochondrial DNA segments (numts)[56,58]. Despite divergence between the mitogenome and numt, mapping only short reads causes an overlap of reads covering approximately 6500-bps of the mitogenome (Appendix C5). To correct for the presence of numts, a consensus of the reads that deviate from the mitogenome reference, created by Dr. Laura Bertola, was added to the reference genome as a representative numt scaffold.

Mitogenome SNP Identification

The mitogenome was isolated from genomic reads and filtered to produce a panel of polymorphic sites for further analysis. BAM files were filtered with a mismatch threshold of 0.03 using SAMtools[183]. Variants were called for the Mitogenome via GATK[184] HaplotypeCaller tool then filtered for a minimum depth of 10-bp, retention of only biallelic SNPs, and removal of

heterozygotes using VCFtools[185] and BCFtools[183]. FASTA sequences were created for individuals containing <10% missing data across the mitogenome using BCFtools. The resulting sequences were combined with published modern mitogenomes from GenBank (KP001493-KP001506[31], KP202262[58], KC834784[27]) and aligned using the EMBL-EBI web tool Clustal Omega (www.ebi.ac.uk/Tools/msa/clustalo). The mitogenome of modern sample M_ZAM_T256 was annotated using the MITOS webserver[186].

Polymorphic sites were identified using dnaSP[187]. To account for potential biases produced by differences in sequencing methods between studies, the multiple sequence alignment (MSA) was trimmed to include only polymorphic sites present in mitogenome sequences generated in this study.

Analysis

Diversity analyses of the MSA were done using PLINK v1.9[188], Arlequin v3.5[116], and DnaSP v6[187]. Principal components analysis (PCA) was done using R package SNPRelate[189] through calculation of Eigenvectors and visualized using the plot3D function in the rgl R package. A median-joining haplotype network was created using POPArt[123] and an unrooted maximum likelihood (ML) tree was inferred in RAxML using a rapid bootstrap with 1000 replicates evaluated under the GTRGAMMAI substitution model[118].

Table 5.2. Historical versus modern genetic diversity for nDNA and mtDNA. N = Sample Size, HE = Expected Heterozygosity, A = Allelic Richness, PA = Private Alleles, M = Garza-Williamson Index, s = Segregating Sites, π = Nucleotide Diversity, Hd = Haplotype Diversity, H = Number of Haplotypes, PM = Private Mutations. Trend is based on statistical significance from a comparison of means. H_E, A, PA, and M had a p-value < 0.005 indicating a downward trend (↓) from historical to modern with a. The p-value for π and Hd was > 0.05 indicating maintained diversity (→) from historical to modern.

	Historical	Modern	Trend
nDNA	*N* 143 H_E 0.833 *A* 15.0 *PA* 6.2 *M* 0.41	*N* 135 H_E 0.796 *A* 11.6 *PA* 1.2 *M* 0.32	↓
mtDNA	*N* 102 *s* 280 π 0.222 *Hd* 0.98 *H* 74 *PM* 22	*N* 19 *s* 258 π 0.258 *Hd* 0.98 *H* 17 *PM* 1	→

RESULTS

Nuclear Analyses

After data calibration, the complete MD contains 135 lions from 14 sampling locations (Table 5.2, Appendix B.1.b). Excluding samples with unknown location or of captive origin, the HD consists of 143 of the 162 lions. The HD has an average of 90% coverage across the nine loci. Sampling is similarly distributed across the lion range for both the MD and HD (Figure 5.1).

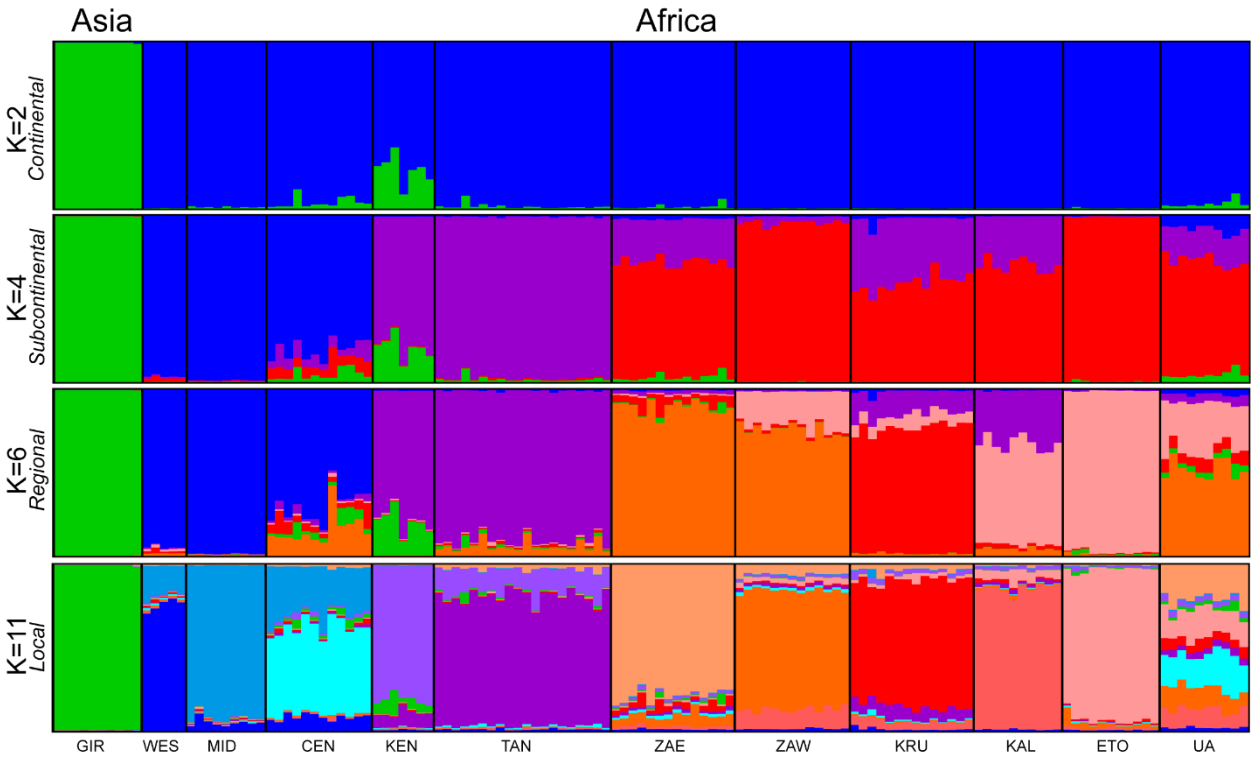


Figure 5.3. The four tiers of population structure as determined by hierarchical structure analysis. Groups are colored based on Figure 5.2.

Fine structure was found in the MD but not the HD. The MD hierarchical structure analysis resulted in four tiers of structure, Continental (K=2), Subcontinental (K=4), Regional (K=6), and Local (K=11), as seen in the final analysis with location priors (Figure 5.3). Appendix C4 shows a graphical display of the step-by-step hierarchical population structure. The initial run had a ΔK of 2 separating Asia (GIR) from Africa. The African population could then be broken down into a Western, Eastern, and Southern population from a ΔK of 3. The Western population also had a ΔK of 3 separating a West African (WES) population, Central African (CEN) population and population between the two (MID). The Eastern population had a ΔK of 2 separating lions in Kenya (KEN) from all lions sampled in Tanzania (TAN). The Southern population is separated into 5 local populations that can be grouped into 3 regional populations

as ΔK was 5, however, there was a sizable peak also seen at K=3. Eastern and western Zambia (ZAE and ZAW) make up a Southeast population while Etosha and Kalahari (ETO & KAL) make up a Southwest population. Kruger can be identified as its own population at both K=3 and K=5. Population clustering in the MD PCoA mimics that of the Subcontinental tier (Figure 5.4). Table 5.3 shows diversity across tiers. Mean heterozygosity across polymorphic loci (H_E) is lowest in GIR and highest in CEN although only 44% of loci are polymorphic.

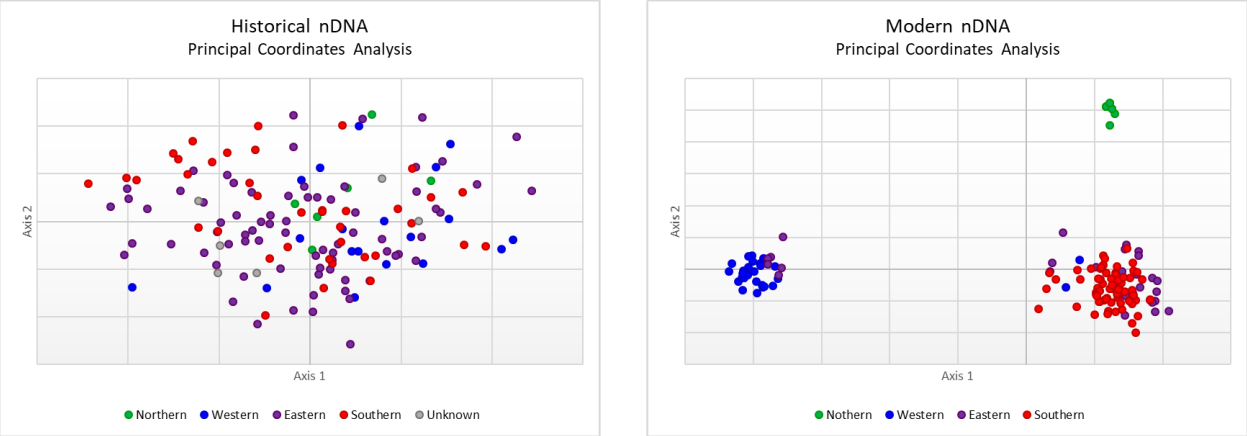


Figure 5.4. Results of a principal coordinate analysis (PCoA) of 9 microsatellite loci.

Structure analysis did not identify any population structure in the HD. While ΔK was also 2 for the initial run, individuals could not be assigned to meaningful populations. Further evidence of this lack of structure can be seen in the PCoA (Figure 5.4). Genetic structure can be identified when populations are forced by grouping based on sampling location (Figure 5.5). The modern branches having the longest branch lengths, with the modern Southern and Eastern groups most similar to the historical Southern group and modern Western and Northern groups most similar to the same historical groups.

Table 5.3. Nuclear genetic diversity for subpopulations defined during hierarchical structure analysis of the modern population. Color is based on when the population emerges during hierarchical structure analysis (see Figure 5.3). Dark gray encompasses all data. Light gray is the continental tier. Purple is the subcontinental tier. Blue is the regional tier. Green in the local tier.

	N	P (%)	A (AP)	Range	H	H_O	H_E	M
All Data	**135**	**100**	**11.6**	**30.0**	**0.80**	**0.64**	**0.80**	**0.32**
Asia	10	22	1.3 (2.5)	6.0	0.09	0.36	0.42	0.05
Africa	125	100	11.6	30.0	0.79	0.69	0.79	0.39
Western	28	100	5.6	19.5	0.74	0.61	0.74	0.18
WES	5	33	2.3 (3.0)	18.7	0.53	0.93	0.79	0.09
MID	9	67	2.0 (2.5)	13.7	0.46	0.76	0.69	0.07
CEN	14	44	3.6 (8.0)	24.5	0.36	0.73	0.82	0.13
Eastern	29	100	6.1	20.9	0.66	0.62	0.66	0.22
KEN	7	33	1.3 (3.7)	16.7	0.22	0.71	0.66	0.05
TAN	22	100	6.1	20.9	0.66	0.63	0.66	0.22
Southern	68	100	9.7	28.0	0.79	0.71	0.79	0.36
Southeast	34	100	7.7	26.4	0.76	0.71	0.76	0.26
ZAE	17	100	6.1	25.1	0.67	0.65	0.67	0.21
ZAW	17	100	6.1	22.4	0.77	0.76	0.77	0.22
South	14	100	6.9	24.0	0.78	0.80	0.78	0.24
Southwest	20	100	5.3	22.9	0.72	0.66	0.72	0.19
KAL	10	100	4.1	16.4	0.69	0.69	0.69	0.15
ETO	10	100	3.8	18.2	0.62	0.63	0.62	0.14

sample size (N)
percentage of polymorphic loci (P)
mean number of alleles per locus (A)
mean number of alleles per polymorphic locus if different than A (AP)
allelic range (Range)
genetic diversity of mean expected heterozygosity per locus (H)
mean observed heterozygosity per polymorphic locus (H_O)
mean expected heterozygosity per polymorphic locus (H_E)
Garza-Williamson modified index (M)

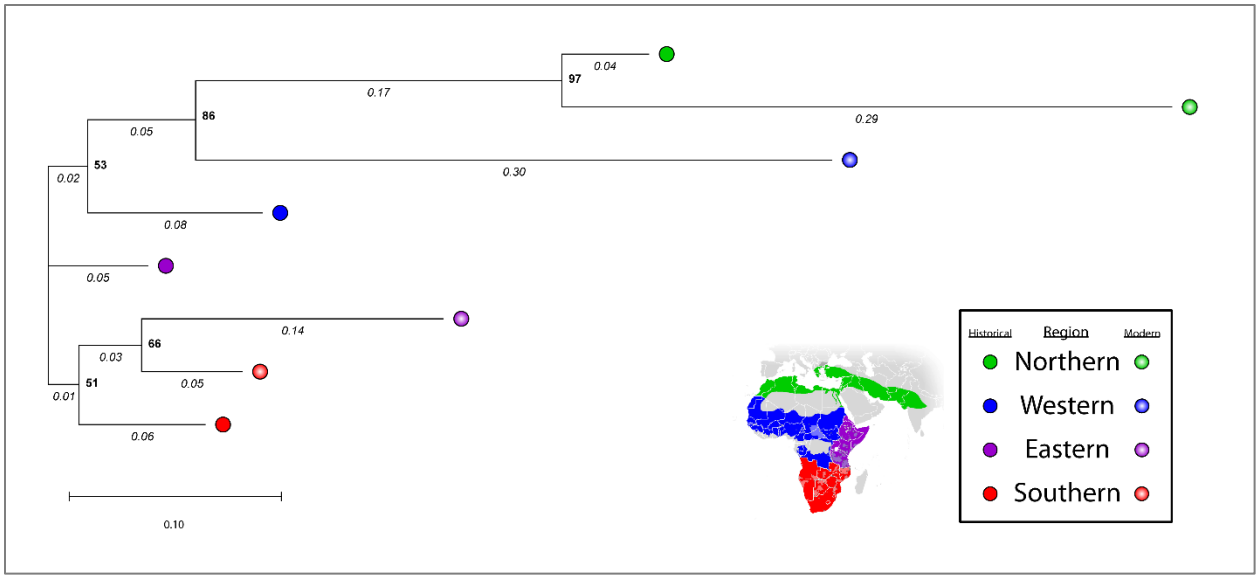


Figure 5.5. Phenetic tree based on D_A genetic distance of microsatellites of conventionally recognized regions.

A significant decrease (p-value < 0.005) from HD to MD is evident across diversity indices (Table 5.2). Correcting for sample size through rarefaction, the HD has an allelic richness of 14.2 and private allelic richness of 4.6, higher than the MD at 11.3 and 1.7, respectively. The Garza-Williamson index (M) of the HD is 0.41 and the MD is 0.32.

Whole Genome Sequencing

Whole genome sequence coverage ranged from 1.3x-9.6x with mitochondrial coverage ranging from 1.9x-1703.9x (Appendix B.1.e). After filtering, there were 102 historical lions and 3 modern lions of sufficient quality for analysis. With the addition of the sequences from GenBank there are a total of 19 modern lions. The annotation of M_ZAM_T256 mitogenome can be found in Appendix C6.

Mitochondrial Analyses

The data were reduced to 280 polymorphic sites identified across the historical lion sequences. The modern population had 258/280 polymorphic sites. Nucleotide diversity (π) is 0.222 for historical and 0.258 for modern. Haplotype diversity (Hd) is 0.98 for both. Eighty-nine haplotypes were identified. The historical population has 74 haplotypes with 22 private mutations while the modern population has 17 haplotypes with only 1 private mutation. Two haplotypes (Hap_33 and Hap_66) are in both populations.

Mitochondrial genome analyses identify 4 major clades, Southern, Mixed, Eastern and Western (Figures 5.6-5.8) with at least one of the 19 modern lions represented. The Western clade includes the Northern subclade. Only bootstrap values ≥70% are reported in the ML tree (Figure 5.6) indicating well supported nodes[190] which equate to these four clades. The 4 clusters in the PCA (Figure 5.7) and the four main branches of the haplotype network (Figure 5.8) also coincide with these clades.

The Southern clade includes the conventionally recognized regions of Southern Africa incorporating Botswana, South Africa, Namibia and Zimbabwe. Botswana and South Africa have haplotypes in both the Southern and Mixed clades. The Mixed clade consists of haplotypes from the Southern, Eastern, and Western subcontinental groups. Zambia and Malawi present exclusively in the Mixed clade, while all other represented countries are also found within other clades. The Western clade includes countries in the conventionally recognized regions of Central and Western Africa including present day Democratic Republic of Congo, Benin, Central African Republic, and Cameroon. The Eastern clade is primarily historical lions from British East Africa, present day Kenya, and a modern lion from Somalia.

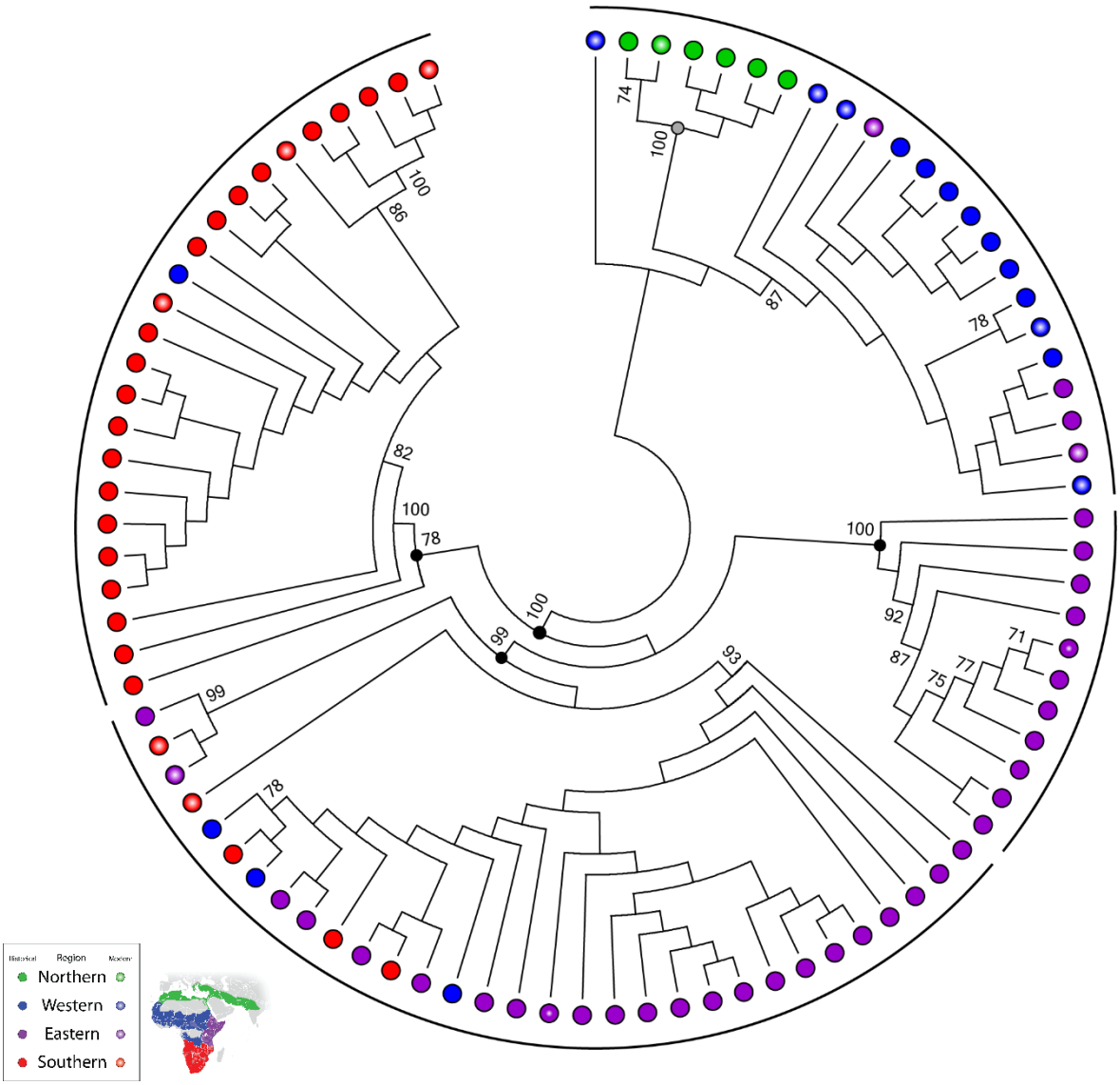


Figure 5.6. Maximum likelihood tree showing nodes with >70% bootstrap support. Black dots denote the nodes of the four major clades. Arcs indicate clade boundaries. The hollow dot denotes the nested Gir Forest clade. Color corresponds to sampling location.

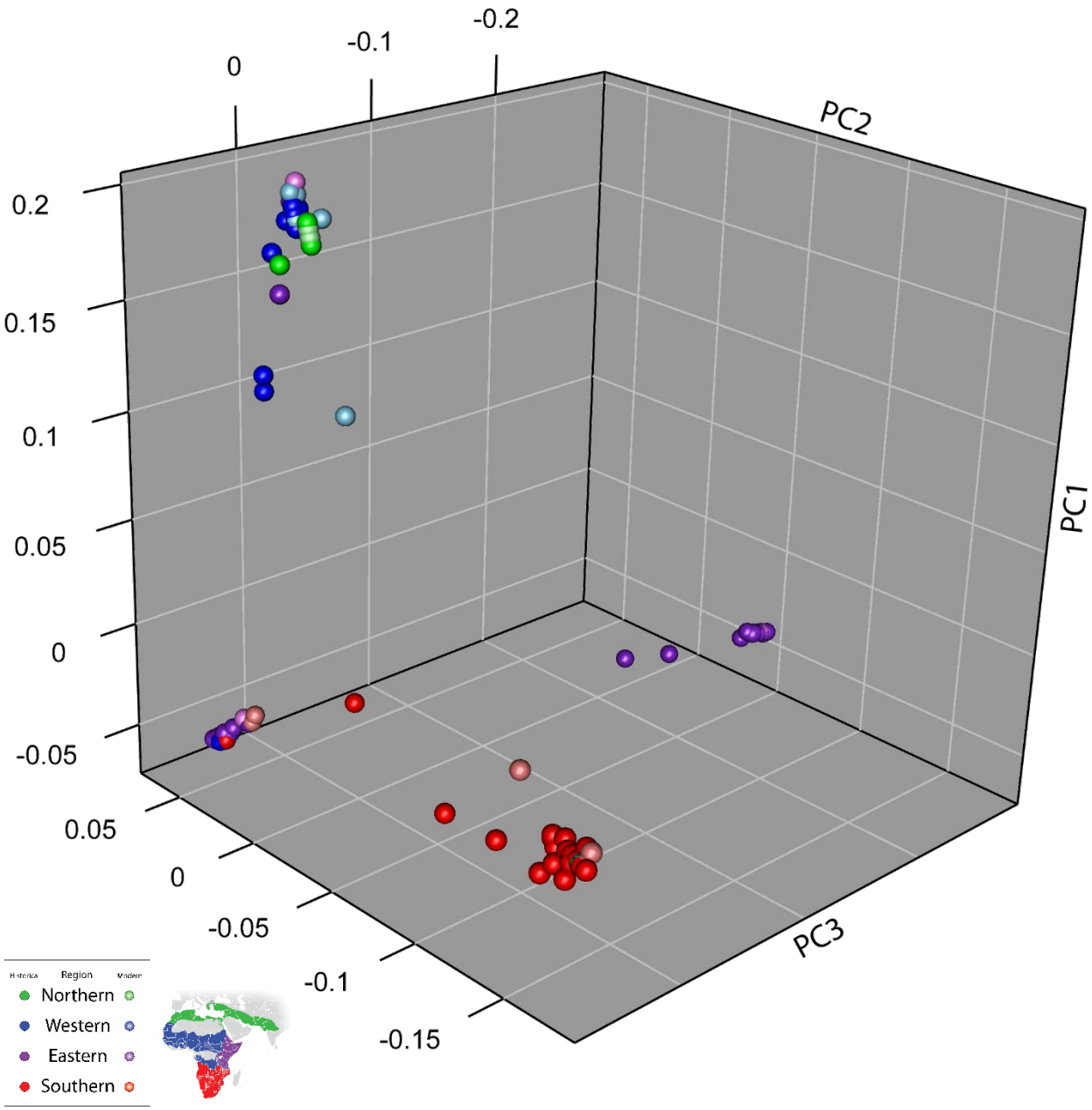


Figure 5.7. Principal Component Analysis of 121 lion mitogenomes. PC1=17%, PC2=12% and PC3=10% of the total variation. Color corresponds to sampling location.

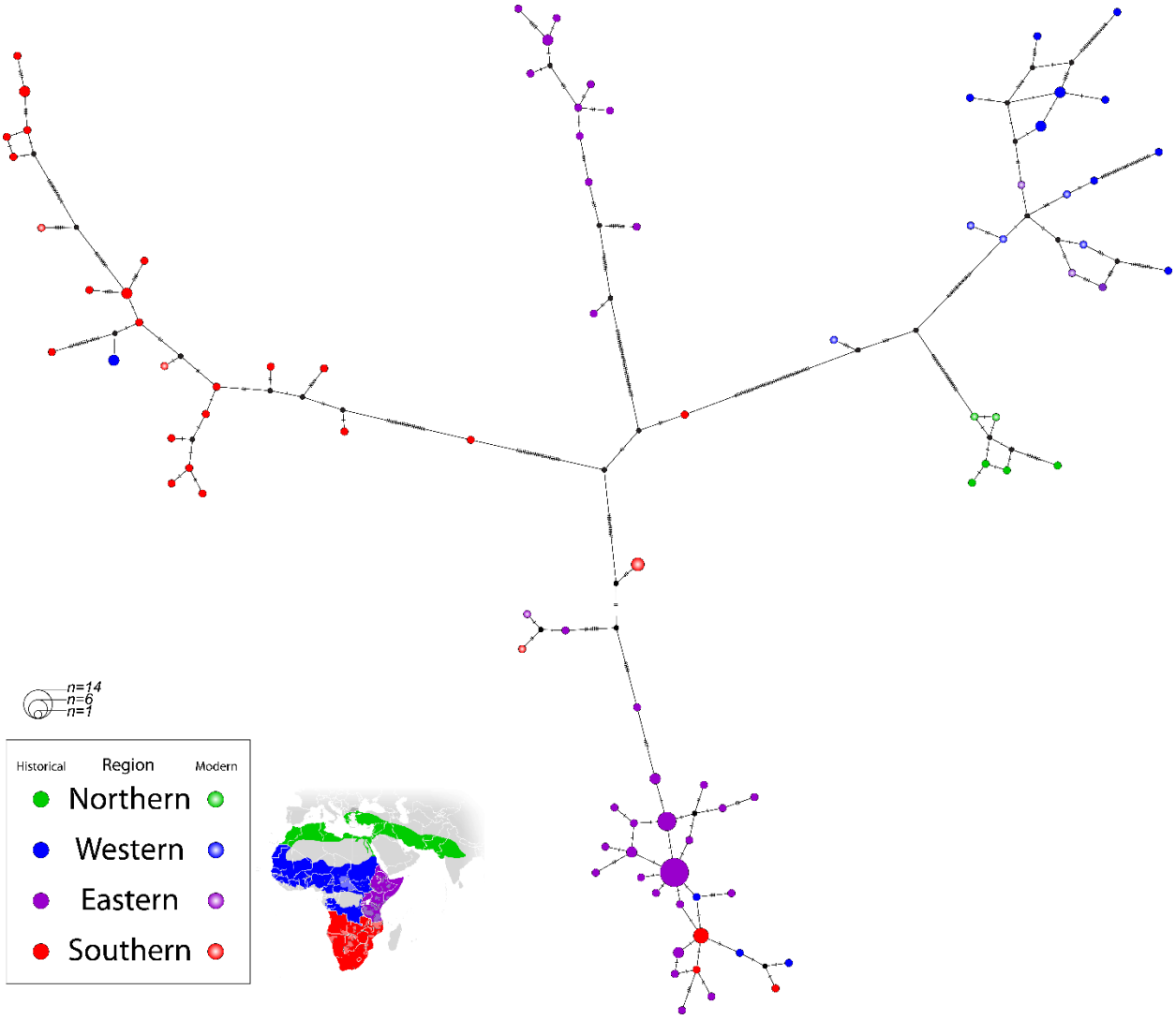


Figure 5.8. Neighbor-joining haplotype network. Color corresponds to sampling location.

DISCUSSION

nDNA

The goal of structural analysis is to provide the smallest value of K that captures the major structure in the data[150]. The Evanno Method[152] calculates ΔK, the uppermost hierarchical level of structure. Secondary peaks in the ΔK plot can indicate hidden fine structure. Knowing that the signal differentiating Africa and Asia is strong[41] and that structure has been found regionally[15,38,41,46,72,75,104,177], we implemented a hierarchical strategy to uncover any hidden fine structure that may be lost when all samples are analyzed together.

Four tiers of modern genetic structure were identified through hierarchical structure analysis. Continental structure separates Asia from Africa. This is the ΔK identified for the entire population by the Evanno Method[152], in agreement with the strong signal found by Bertola *et al* 2015[41]. The subcontinental tier identifies three main groups in Africa: Western, Eastern, and Southern. Smitz *et al* 2018[48] identified only two groups at the continental scale. Lack of identification of a Southern group was likely due to low sampling in the south. The regional tier divides the Southern group into a Southwest, South, and Southeast group. The highest level of population structure was able to detect most sampling locations. Only sampling locations in Tanzania couldn't be individually identified. This corresponds with Smitz *et al* 2018[48], which found no structure at the Tanzanian scale.

There is admixture evident within local groups (Figure 5.3, K=11) indicating recent gene flow. The UA group is comprised of individuals sampled within local groups who could not be assigned to a particular tier due to admixture. Other range-wide studies have shown a similar localized structure pattern with individuals assigned to sampling populations with evidence of isolation-by-distance[15,38].

Nuclear studies show both high[42,48,76] and low[46,104,177] levels of gene flow depending on the amount of connectivity present between sampling locations. Lack of connectivity has isolated populations such as those in the Kainji Lake National Park from Yankari Game Reserve in Nigeria[46] and Kafue National Park from the Luangwa Valley Ecosystem in Zambia[104]. Genetic differentiation can even be seen between populations within national parks[177]. However, where there are no geographic or artificial barriers to limit movement, there is only weak evidence of population structure and high levels of gene flow[48,76].

Historically, the lion range was more continuous and connected. The *Scramble for Africa* in the late-1800's increased European rule from 10% to 90% of the African continent[168]. Increased European settlement inevitably lead to an exponential increase in the human population, urban development, and rural expansion[167] resulting in changes to the African landscape and fragmentation to the lion's current range[191].

Habitat fragmentation restricts gene flow and the more isolated a population becomes the more likely it is to lose genetic diversity[192,193]. The KAZA has seen a decrease in genetic and allelic diversity in the past century[49]. This study shows there has been a significant decrease in nDNA diversity from the range-wide historical to the modern lion population (Table 5.2). Expected heterozygosity (HE), allelic richness (AR), and number of private alleles (PA) have all significantly decreased (p-value < 0.0005). And, while there is evidence of a recent reduction in size in both the historical and modern populations both displaying an M value of <0.67[194] (Table 5.2), M is significantly lower in the modern population. This indicates the reduction predates the *Scramble to Africa* but has increased in the past century.

While lions currently exhibit fine population structure, the historical population lacked any population structure, suggesting lions were a panmictic population only a century ago. This

was confirmed through structure analysis and PCoA. The modern population shows clusters distinguishing the four subcontinental groups while there is no distinction between groups in the historical population, not even between Asia and Africa. Though, the phenetic tree showed the modern groups are most similar to their historical counterparts (Figure 5.5). This suggests regional similarities over time when subpopulations are forced based on sampling location and indicates the direction of recent divergence.

With the strong signal differentiating the Asiatic and African lions in the modern population, the historical lions from the Gir Forest NP were expected to cluster independently of the historical African lions. The PCoA, however, shows these lions cluster together in the center of a single historical lion cluster (Figure 5.4). Lions were at the brink of extinction in Asia at the beginning of the 20th century[195] when these samples were collected (1906-1929). Today there are over 400 lions in the Gir Forest NP. The historical and modern samples from the Gir Forest NP were collected at the peak of a recent bottleneck and its subsequent population restoration. From this comparison we are able see this severe bottleneck has resulted in low genetic diversity in Asia compared to Africa (Table 5.3). Habitat fragmentation leading to the isolation of subpopulations within Africa is following the same trend as the Asiatic lion a century ago.

mtDNA

Historical and modern mitogenomes tell another story. Although nuclear diversity has significantly decreased, mtDNA diversity has remained constant over time (Table 5.2). Mitochondrial DNA is matrilineally inherited demonstrating female-mediated gene flow. Localized studies show there is little or no female-mediated gene flow between subpopulations[45,91,104]. In the lion mating system, females primarily remain with their natal pride

while males disperse[9,196]. Therefore, pride structure can dictate mtDNA population structure. With females remaining close to their natal prides, habitat fragmentation does not greatly alter pride structure, keeping mtDNA diversity constant over time.

Polymorphic sites were restricted to those found in the sequences generated in this study. Conservative filtering could have missed variation in the historical mitogenomes that are present in the published mitogenomes. Therefore, polymorphic sites found in the published mitogenomes were excluded to reduce potential biases produced by differences in sequencing between studies.

There was no significant difference found between mtDNA π and Hd. The low number of private mutations (PM) in the modern population is likely a result of the small number of mitogenomes compared to the historical population. While we were able to get a large number of historical mitogenomes from our museum samples, the number of modern mitogenomes was restricted to availability of published mitogenomes. As next generation sequencing keeps getting more affordable and more modern mitogenomes become available, these analyses could be repeated with larger sample sizes for more power.

The distribution of modern haplotypes within clades is geographically consistent with historical haplotypes (Figures 5.6-5.8). The four major clades (Southern, Mixed, Eastern, and Western) geographically follow the subcontinental groups identified by nDNA analysis (Southern, Eastern, Western, and Northern). When compared to previously published haplotype networks[15,41,104] (Figure 5.9), the four major clades can be identified illustrating maintenance of mitochondrial structure over time.

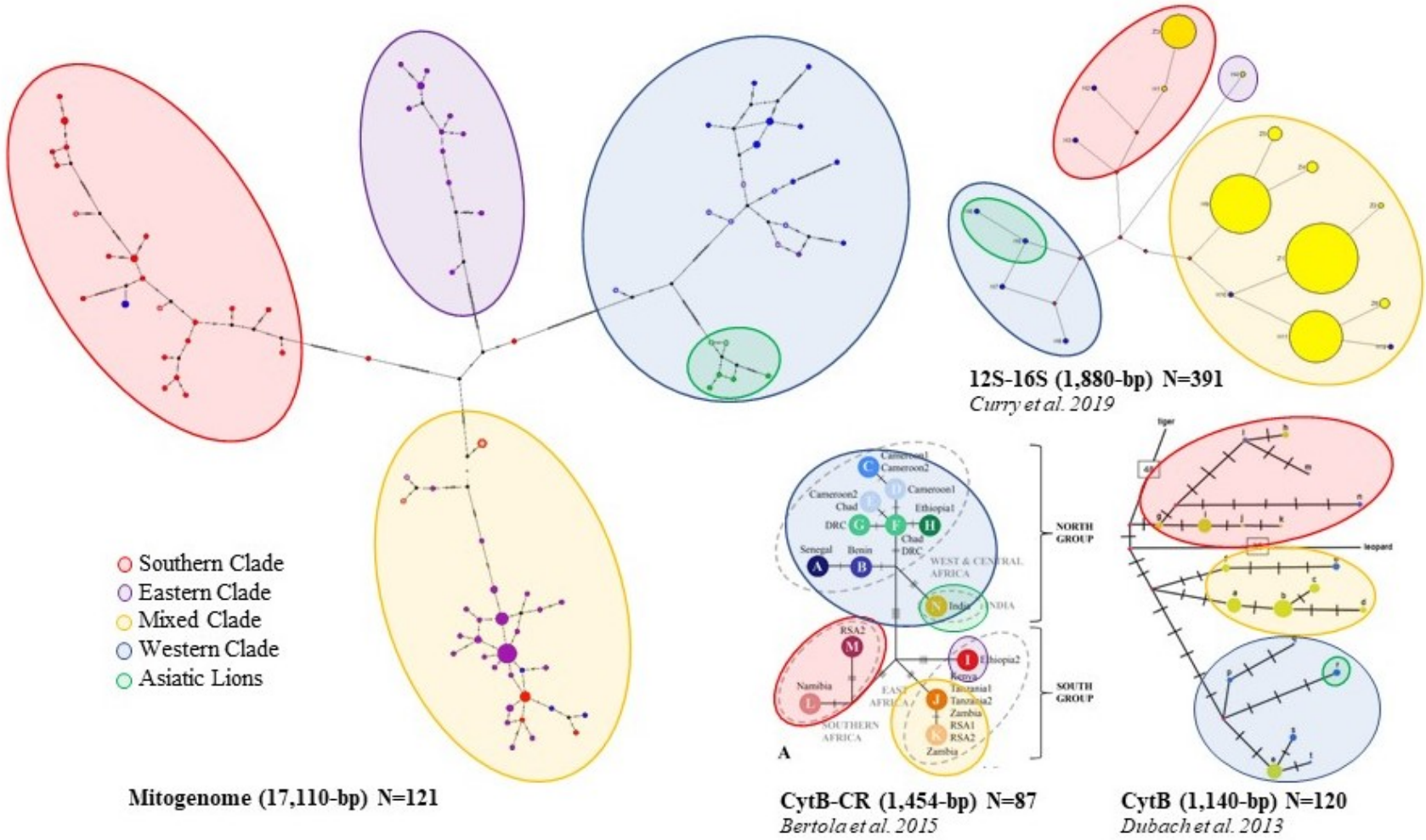


Figure 5.9. Comparison with previously published haplotype networks illustrating maintenance of mitochondrial structure.

The Western clade includes West and Central Africa as well as Asia. Previous studies have suggested that the Asiatic and West African lion should be grouped taxonomically[14,31]. USFWS recognizes these two as the same subspecies under the Endangered Species Act (ESA)[24]. Mitogenome analyses support this claim placing the Gir Forest NP lions within the Western clade in all analyses (Figures 5.6-5.8). Eastern haplotypes within the Western clade are from bordering countries suggesting gene flow between neighboring regions.

The Mixed clade is an intermediary clade consisting of lions from Southern and Eastern Africa. The historical samples from the Congo Region (present day Republic of the Congo and Gabon) were assigned to the Western subcontinental group by convention but mitochondrial analyses consistently clustered them within the Southern and Mixed clades. The Congo Region is below the Congolese rainforests, a geographical barrier isolating them from West and Central Africa to the north. For the lion, the Congo Region is, therefore, closer to East and Southern Africa.

Male-Mediated Gene Flow

A comparison of nDNA and mtDNA analyses between historical and modern datasets indicate the presence of male-mediated gene flow with evidence of recent isolation of local subpopulations due to habitat fragmentation.

In the lion mating system, females primarily remain with their natal pride while males disperse[9,196]. If unobstructed by geographic or artificial barriers, a male lion's home range can be hundreds to even thousands of sq. km[197–199] and can span different habitats[200–202]. However, the range of the lion has been greatly reduced as a direct result of the growing human population[19]

and changes in land-use including an expansion of large-scale cultivation and increased movement of livestock into protected areas[203].

The dichotomy between the nuclear and mitochondrial structure is indicative of male-mediated gene flow and female philopatry. This dichotomy is not as evident in the modern population as population fragmentation has hindered the ability of males to cross between isolated subpopulations. This dichotomy could only be seen through a comparison of historical and modern datasets for nDNA and mtDNA.

A century ago, the lion population was a continuous panmictic population consisting of close proximity prides. As lion habitat has become more fragmented and groups of prides become more isolated, gene flow is restricted and subpopulations become more distinguishable from each other genetically. Connectivity is a critical component to enable gene flow between subpopulations to avoid consequences of habitat fragmentation such as the erosion of genetic diversity[173]. As an iconic species, the lion is also a flagship species. These results are an indicator exposing the influence of fragmentation, likely also affecting hundreds of other species. We are already seeing the beginning effects of habitat fragmentation in lions through increased nDNA structure and decreased nDNA diversity. Proper management can prevent the loss of nDNA diversity and ensure mtDNA diversity continues to be maintained. Intervention is needed to increase gene flow between subpopulations and reduce the effects of habitat fragmentation.

CHAPTER VI

CONCLUSIONS

IMPROVING TECHNOLOGIES

Cross-species amplification using domestic cat microsatellite primers is the primary method used for nuclear genetic research in lions (Panthera leo). Genetic differences introduced over 10.8 million years of divergence between the two species make these markers problematic when using low quality and low quantity DNA, a common issue in wildlife genetic research. To increase amplification success of microsatellites in the lion, miniSTRs (<150 bp) with primers designed to be closer to the target region and specific to the lion were developed. Lion specific STRs were successfully designed for 14 of 17 commonly used microsatellites, 10 of 14 being miniSTRs, all with 100% amplification success when tested on 30 lion samples with DNA of varying quality and quantity.

ZAMBIA: A BRIDGE DESPITE BARRIERS

The determination and characterization of lion subpopulations represent a higher-resolution of knowledge regarding both the genetic health and connectivity of the overall lion population, which can serve to inform conservation and management.

Genetic analysis of sequence diversity of 165 African lions from five main areas in Zambia at the 12S to 16S mitochondrial genes uncovered haplotypes which link Southern Africa with East Africa. However, based on an AMOVA analysis, there is little to no matrilineal gene flow (F_{ST}=0.47) between an eastern and western regional subpopulation. These sub-populations live in different habitats and are separated by a large city surrounded by farmland and were,

therefore, suspected to be completely isolated from each other. Nuclear and mitochondrial DNA results from 409 lions then further supported this population substructure across Zambia but proposed only partial isolation with more movement between subpopulations than previously thought. We found genetic evidence that small numbers of lions are moving across these areas thought to be uninhabitable by lions.

Eight haplotypes were found throughout Zambia; three haplotypes previously described and the remaining five novel. The addition of these five novel haplotypes nearly doubled the number of haplotypes previously reported for any given geographic location of wild lions. The addition of microsatellite analysis suggests there is gene flow (F_{ST}=0.04) with low but significant isolation-by-distance and an average of 6 migrants per generation. Gene flow is occurring through the southern regions of the eastern sub-population when lions are moving between the Lower Zambezi National Park and eastern corridor to/from Kafue National Park. Finally, phylogenetic analysis suggests Zambia may serve as a bridge connecting the lion populations in southern Africa to eastern Africa, supporting earlier hypotheses that eastern-southern Africa may represent the evolutionary cradle for the species.

A CENTURY OF CHANGE

The *Scramble for Africa* in the late 1800's was the beginning of a time of increased population growth in Africa. Here, we determined the genetic architecture of both historical (>100 years ago) and modern (2000 to present) lions to identify the extent of change in genetic diversity that has occurred in this time of landscape and anthropogenic change. The historical lion dataset is DNA isolated from high-quality and well-documented museum specimens while the modern lion dataset is data from recently published studies. Analysis of 9 microsatellites

($N_{Historical}$=143, N_{Modern}=135) and 280 polymorphic sites across the mitogenome ($N_{Historical}$=102, N_{Modern}=19) indicate the presence of male-mediated gene flow and evidence of recent isolation of local subpopulations due to habitat fragmentation. Nuclear DNA shows a significant decrease (p-value <0.0005) in genetic diversity from the historical (H_E=0.833) to the modern (H_E=0.796) population while mitochondrial genetic diversity has been maintained (Hd=0.98 for both). While the historical population appears to be a panmictic population, hierarchical structure analysis identifies four tiers of fine structure in the modern population able to detect most sampling locations. Mitochondrial analyses identified 4 clusters: Southern, Mixed, Eastern, and Western. These clusters are consistent between modern and historical haplotypes. Within the last century, habitat fragmentation has caused lion subpopulations to become more isolated as human expansion changes the African landscape. The result is increased fine nuclear structure with a decrease in genetic diversity as subpopulations become more differentiated while mitochondrial structure and diversity is maintained over time.

FINAL THOUGHTS & FUTURE DIRECTIONS

This study is among the first of its kind in using these technologies for a range-wide study of this iconic wildlife species. The goal of this study was to provide the best scientific information available to allow policy makers to make informed decisions for the long-term preservation of the lion.

The differences evident between the historical and modern datasets brings about various possible conservation implications. Historically, lions were a continuous population but habitat fragmentation is creating subpopulation structure. If left unattended, these subpopulations could become completely isolated leading to further differentiation. Managing a species as a

continuous population when they don't have a continuous habitat would require a lot of resources. Lions currently reside in 28 countries. Country by country differences in policy could complicate range-wide management[204] and act as additional artificial barrier. Cooperative management across these countries would be needed to attempt returning the lion population to its historical state. Currently, the African Lion Working Group recommends using regional guidelines for sourcing lions for translocations[205]. Strick guidelines may not be as critical for maintaining the populations' genetic diversity if the goal is based on the historical state.

Lions are a flagship species, meaning that as a large, charismatic carnivore, their research and conservation influences many other species that share its habitat. Knowing the connectivity and levels of gene flow throughout the population and how it has changed over time helps in making decisions on where and how to manage lions and other wildlife populations.

While there have been studies on various subpopulations of lions, the overlap of techniques and technologies used is minimal, making comparison between studies difficult. This study used the same techniques and technologies range-wide across time to make a direct comparison to assess the level of change brought on by a changing landscape. However, not all subpopulations have been sampled. To properly determine the amount of connectivity and levels of gene flow in the current population, lions need to be sampled from each of the fragmented populations. A genetic study needs to be conducted with even larger sample sizes and wider coverage to better understand substructure across the full range of the lion. The creation and curation of a lion genetic database will also benefit lion conservation by allowing future research to continue to build on current knowledge. To accomplish this goal, collaboration across research groups is needed. This will allow us to investigate how genetic diversity is and can further be maintained.

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APPENDIX A

PROTOCOLS

1. Sample Preparation & DNA Extraction

    a. Modified Qiagen DNA Isolation from Tissue
    b. KAPA Express Hair Extraction Protocol
    c. Protocol for DNA Extraction from Skeletal & Tissue Remains
    d. Qiagen DNeasy Purification of Total DNA from Animal Tissues Protocol

2. PCR & Genetic Analyzer Prep

    a. FCA Primer PCR Protocol w Temperature Gradient
    b. 3130/3730 Plate Setup for Fluorescently Labeled PCR Products
    c. Final Leo STR Multiplex PCR Protocols
    d. PCR Protocol for mtDNA Genes 12S-16S
    e. BigDye Terminator & XTerminator Purification for Sequencing

A.1.a: MODIFIED QIAGEN DNA ISOLATION FROM TISSUE

Part 1

1. Cut a small piece of tissue into a 1.7-mL tube for each sample
2. Add 300-µL Cell Lysis Solution to each tube
3. Add 5-µL Proteinase K to each tube
4. Vortex and incubate at 56ºC overnight or until samples have lysed

Part 2

1. Place tubes on ice for 7min
2. Add 100-µL of protein precipitation solution
3. Vortex 20 sec (keep finger on the cap as they tend to open after ice) and spin for 3 min
4. Transfer supernatant to new tubes and precipitate DNA with an equal amount (≈300-µL) of 100% Isopropanol
5. Invert tubes 50x and spin for 3 min
6. Discard supernatant and wash DNA with 300-µL 70% Ethanol
7. Spin 2 min and discard supernatant
8. Allow to air dry and rehydrate DNA with 50-µL 1xTE

A.1.b: KAPA EXPRESS HAIR EXTRACTION PROTOCOL

1. Rinse hair follicles with 70% ethanol and blot dry with kimwipe.
2. Cut 1 hair follicle into 0.2-mL tube.
3. Spin tubes briefly before starting extraction to ensure all follicles are at the bottoms of the tubes.
4. Thaw 10x Buffer, mix reagents as follow: (Mix 4 to 5 sample extra)
    - 10xBuffer 2-µL
    - Express Enyzme 0.2-µL
    - H_2O 17.8-µL
    - Total 20-µL
5. Vortex buffer mixture & spin down briefly.
6. Transfer 20-uL mixture into 0.2-mL tubes containing hair follicles.
7. Vortex tubes & centrifuge briefly. Hair follicles MUST be in the solution before the incubation.
8. Place on thermal cycler at 75°C for 10 min, 95°C for 5 min.
9. Vortex tubes 5 sec & Spin at full speed (3500 rpm=4500g) in Hermle plate centrifuge for 2 min.
10. Transfer entire liquid portion to 0.65-mL Eppendorf tubes or into 96-well plates, leaving behind hair follicles. If needed, samples can be covered at this point and stored at 4°C overnight.

A.1.c: PROTOCOL FOR DNA EXTRACTION FROM SKELETAL & TISSUE REMAINS

*Modified from UNT Center for Human Identification Procedures**

*Ambers A, Gill-King H, Dirkmaat D, Benjamin R, King J, Budowle B. Autosomal and Y-STR analysis of degraded DNA from the 120-year-old skeletal remains of Ezekiel Harper. Forensic Sci Int Genet [Internet]. 2014;9(1):33–41. Available from: http://dx.doi.org/10.1016/j.fsigen.2013.10.014

EQUIPMENT & SUPPLIES

- 15ml polypropylene conical tubes
- 56^{0C} orbital shaker
- Amicon Ultra-15 Centrifugal Filter Device – 30K
- Centrifuge for 15mL tubes
- (2) 1.7mL microcentrifuge tubes
- Qiagen QIAquick DNA purification spin columns

REAGENTS

- Sodium N-Laurylsarcosinate (aka sarkosyl)
- 0.5M EDTA (8.0 pH)
- Proteinase K (20mg/mL)
- Phenol Chorophorm Isoamyl Alcohol (25:24:1) = PCIA 25:24:1
- TE^{-4} *(or ddH_2O)*
- Qiagen QIAquick Buffers & Wash Solution
    - PB – Binding Buffer
    - PE – Wash Buffer
    - EB – Elution Buffer

PREPARATION

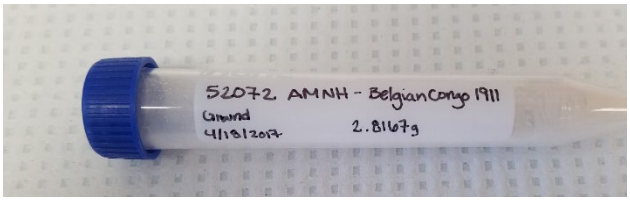
- Clean work surfaces, tube racks and equipment with 10% bleach, ddH_20 and 95% EtOH.
- UV crosslink work areas, reagents and plastics for 1hr before use.

Prepare Demineralization Buffer:

- Add 5g Sodium N-Laurylsarcosinate to 500mL of 0.5M EDTA (pH 8.0)
- Swirl to dissolve
    - Good for 1-yr

LABELING EXAMPLES

- 15ml polypropylene conical tubes



- Final 1.7mL microcentrifuge tube for DNA storage

PROCESS SKELETAL REMAINS (BONE & TEETH)

Process samples in the "Clean" Workstation (C.B.S Scientific Model #P-048-202) one at a time.
Between each sample: clean Workstation with bleach, ddH_2O and EtOH. Run UV light for < 20 min.

Bones, Bone Fragments & Teeth:

- **Clean**
  - Rinse and spot clean sample with 95% EtOH.
  - Large samples – remove outer layer of bone with DREMEL® Sanding Band (120 grit: Product #430).
  - Small samples – remove outer layer of bone with SeaSky dentistry drill fit with a diamond bur.
  - Wipe clean with 95% EtOH.
  - UV irradiate sample for 5 min.
- **Dry** samples completely before cutting or grinding.
- **Cut** *(if necessary – larger samples only)*
  - Use DREMEL® Heavy Duty Cut-off Wheels (Product #420) to cut larger samples into smaller pieces (≈¼ inch (6 mm)).
  - Cut in 3-second interval as not to expose sample to too much heat and degrade DNA.
  - Cut over a large petri dish or weigh boat to collect dust and fragments while cutting.
- **Collect** fragments and shavings into a grinding tube fitted with end caps and impactor added.
- **Grind** sample using Spex 6750 Freezer Mill "Bone" protocol.
  - After grinding, ensured entire sample is powder. If not, run additional sequence.
- Allow grinding vial to come to room temperature (may take up to 8 hours).
- **Transfer** powder into a pre-weighed 15-mL conical tube. Record weight of powder.
- Proceed to **DNA Extraction** Part 1

  OR
- **Store** in storage room (VRB 226A) @ room temperature for interim storage

Turbinates, Porous Bone & Fragments too small to grind:

- **Clean** by UV irradiation in a weigh boat for 5 min.
- **Collect** fragments into a pre-weighed 15-mL conical tube. Record weight.
- **Crush** fragments with forceps.
- Proceed to **DNA Extraction** Part 1

  OR
- **Store** in storage room (VRB 226A) @ room temperature for interim storage

PROCESS SKELETAL REMAINS (DRIED TISSUE, MUSCLE, CARTILAGE, & HIDE WITH HAIR)

Process samples in the "Clean" Workstation (C.B.S Scientific Model #P-048-202) one at a time.
Between each sample: clean Workstation with bleach, ddH_2O and EtOH. Run UV light for < 20 min.

Dried Cartilage:

- **Clean**
  - Rinse and spot clean sample with 95% EtOH.
  - UV irradiate sample for 5 min.
- **Dry** samples completely before proceeding.
- **Cut** *(if necessary – larger samples only)*
  - Use DREMEL® Heavy Duty Cut-off Wheels (Product #420) to cut larger samples into smaller pieces (≈¼ inch (6 mm)).
  - Cut in 3-second interval as not to expose sample to too much heat and degrade DNA.
  - Cut over a large petri dish or weigh boat to collect dust and fragments while cutting.
- **Collect** fragments and shavings into a grinding tube fitted with end caps and impactor added.
- **Grind** sample using Spex 6750 Freezer Mill "Bone" protocol.
  - After grinding, ensured entire sample is powder. If not, run additional sequence.
- Allow grinding vial to come to room temperature (may take up to 8 hours).
- **Transfer** powder into a pre-weighed 15-mL conical tube. Record weight of powder.
- Proceed to **DNA Extraction** Part 1

  OR
- **Store** in storage room (VRB 226A) @ room temperature for interim storage

Hide with Hair:

- **Clean** by UV irradiation in a weigh boat for 5 min.
- **Cut** *(if necessary – larger samples only)*
  - Use scissors to cut strips small enough to fit into grinding vial.
- **Grind** sample using Spex 6750 Freezer Mill "Bone" protocol.
  - After grinding, ensured entire sample is powder. If not, run additional sequence.
- Allow grinding vial to come to room temperature (may take up to 8 hours).
- **Transfer** powder into a pre-weighed 15-mL conical tube. Record weight of powder.
- Proceed to **DNA Extraction** Part 1

  OR
- **Store** in storage room (VRB 226A) @ room temperature for interim storage

Dried Tissue:

- **Clean**
  - Rinse sample with 95% EtOH.
  - UV irradiate sample for 5 min.
- **Collect** dry, clean tissue in a 1.7mL microcentrifuge tube.
- Proceed to ***Qiagen DNeasy Purification of Total DNA from Animal Tissues Protocol***

  OR
- **Store** in storage room (VRB 226A) @ room temperature for interim storage

DNA EXTRACTION PART 1

- **DAY 1:**
  - **Transfer** 0.5g to 1.0g of powder or crushed bone to a 15mL conical tube
  - **Add** 4.5mL demineralization buffer + 300µL Proteinase K to each sample
    - If < 0.5g bone powder, add 3mL demineralization buffer + 200µL Proteinase K
  - **Incubate** on a 56°C orbital shaker for 16-24hrs* (Parafilm lid to prevent leaking)
    **Exceeding 24hrs (48hr max) will not increase DNA recovery but is acceptable if necessary for workflow.*
- **DAY 2:**
  - **Centrifuge** tubes briefly to settle contents
  - **Add** equal volume PCAI 25:24:1 to conical tube
    e.g. add 5mL PCAI 25:24:1 to 5mL sample solution
  - **Vortex** 30 sec on medium speed
  - **Centrifuge** @ 4,000 rpm (*centrifuge max speed*) for 3min
  - **Transfer** aqueous layer to an Amicon centrifuge filter device
    - ***Optional:*** Transfer aqueous layer to a new 15mL conical tube for an additional PCIA 25:24:1 step before transferring to Amicon if aqueous layer is cloudy
  - **Centrifuge** Amicon @ 4,000 rpm for ≈40 min (until ≈250µL of sample remains in filter)
  - **Add** 2mL TE^{-4} to filter and centrifuge @ 4,000 x g for ≈40 min (until ≈250µL remains)
    - Discard flow-through
  - **Rinse** filter with residual TE^{-4} in Amicon
  - **Transfer** DNA extract (100-200µL) to 1.7mL tube
  - Rinse filter with an additional 100µL TE^{-4} and transfer to the 1.7mL tube
  - Proceed to ***Qiagen QIAquick Purification***
    OR
  - **Store** @ 4°C for interim storage

DNA EXTRACTION PART 2

- **QIAGEN QIAQUICK PURIFICATION FOR INHIBITOR REMOVAL:**
  - **Add** 5 volumes of Buffer PB to 1 volume of sample. Vortex
    e.g. add 1000µL PB to 200µL sample
  - **Add** 650µL PB/extract mixture to QIAquick spin column in 2mL collection tube
    - Centrifuge @ 13,000 x g (*centrifuge max speed*) for 30sec
    - Discard flow-through
    - Repeat until all PB/extract mixture has been filtered through column
  - **Wash** column with 750µL Buffer PE.
    - Centrifuge @ 13,000 x g for 60sec
    - Discard flow-through
    - Centrifuge column @ 13,000 x g for an additional 60sec
  - Place column in a sterile 1.7mL tube and discard collection tube
  - **Elute DNA** (2x)
    - Add 50µL Buffer EB to center column
    - Let sit 1min @ room temperature
    - Centrifuge@ 13,000 for 60sec
    - Repeat above for 2nd elution (final volume of 100µL)
  - Discard spin column
  - **Store** extracts @ 4°C for short term and -20°C for long term storage

A.1.d: QIAGEN DNEASY PURIFICATION OF TOTAL DNA FROM ANIMAL TISSUES PROTOCOL

- **Sample:**
  - Place up to 25 mg tissue cut into small pieces in a 1.7 ml microcentrifuge tube.
- **Lyse:**
  - Add 180 µl **Buffer ATL**.
  - Add 20 µl **proteinase K**.
  - Mix thoroughly by vortexing
  - Incubate at 56°C until completely lysed.
    - *Vortex occasionally during incubation to disperse the sample, or place in a thermomixer, shaking water bath, or on a rocking platform.*
    - *Lysis time varies depending on the type of tissue processed. Lysis is usually complete in 1–3 hrs. If it is more convenient, samples can be lysed overnight; this will not affect them adversely.*
- **Bind:**
  - Vortex for 15 s.
  - Add 200 µl **Buffer AL** and mix thoroughly by vortexing.
  - Add 200 µl **ethanol** (96–100%) and mix thoroughly by vortexing.
    - *It is essential that the sample, Buffer AL, and ethanol are mixed immediately and thoroughly by vortexing or pipetting to yield a homogeneous solution. Buffer AL and ethanol can be premixed and added together in one step to save time when processing multiple samples.*
    - *A white precipitate may form on addition of Buffer AL and ethanol. This precipitate does not interfere with the DNeasy procedure.*
  - Pipet the mixture (including any precipitate) into the **DNeasy Mini spin column placed in a 2 ml collection tube** (provided).
  - Centrifuge at 8000 rpm for 1 min. Discard flow-through and collection tube.
  - Place the DNeasy Mini spin column in a **new 2 ml collection tube** (provided).
- **Wash:**
  - Add 500 µl **Buffer AW1**.
  - Centrifuge for 1 min at 8000 rpm. Discard flow-through and collection tube.
  - Place the DNeasy Mini spin column in a **new 2 ml collection tube** (provided)
  - Add 500 µl **Buffer AW2.**
  - Centrifuge for 3 min at 14,000 rpm to dry the DNeasy membrane. Discard flow-through and collection tube.
  - Transfer the spin column in a clean 1.7 ml microcentrifuge tube.
- **Elute:**
  - Pipet 200 µl **Buffer AE** directly onto the DNeasy membrane.
  - Incubate at room temperature for 1 min.
  - Centrifuge for 1 min at 8000 rpm to elute.
    - Recommended: For maximum DNA yield, repeat elution.
      - A new microcentrifuge tube can be used for the second elution step to prevent dilution of the first eluate. Alternatively, to combine the eluates, the microcentrifuge

A.2.a: FCA Primer PCR Protocol w Temperature Gradient

KAPA2G Robus HotStart PCR Protocol - 25µL Reaction

updated: 11/11/2015 (CJC) VeriFlex™

	AMOUNT
ddH2O	10.900
5X KAPA2G Buffer A	5.000
5X KAPA Enhancer 1	5.000
Forward Primer (10µM)	1.250
Reverse Primer (10µM)	1.250
dNTPs (10mM)	0.500
KAPA2G Robust HotStart Polymerase (5 units/µL)	0.100
DNA (≤20ng/uL)	1.000
TOTAL	**25.000**

95	3m	x1
95	15s	x35
**	15s	
72	45s	
72	1m	x1
4	∞	

**see plate record

96-well Plate

Temp	Row
54°C	1
	2
56°C	3
	4
58°C	5
	6
60°C	7
	8
62°C	9
	10
64°C	11
	12

A.2.b: 3130/3730 Plate Setup for Fluorescently Labeled PCR Products

updated: 10/17/2017 CJC

1. Prepare master mix

	µL ea	# samples:
size standard	0.3	0
DI formamide	10	0
sum	10.3	0

2. Vortex & spin down master mix
3. Divide master mix into 8-well strip tube.
4. Use multichannel P10 pipettor to transfer 10 uL master mix into each well of a 96-well plate.
5. Add PCR product onto 96-well plate using multichannel P10 pipettor.

His	Mix 1	Mix 2	Mix 3	Mix 4	
1.5	1	1	1	0.5	Leo098
				1	Leo230 & Leo506

6. Spin plate briefly to pool liquid and remove bubbles.
7. Add appropriate rubber grommet or septa to top of plate.
8. Denature at 96C for 4 minutes.
9. Place plates immediately on ice block in -20C freezer to load on 3130.

A.2.c: Final Leo STR Multiplex PCR Protocols

KAPA2G Robus HotStart PCR Protocol - 25µL Rxn

updated: 10/17/2017 (CJC) - Mix 1	AMOUNT
ddH2O	8.900
5X KAPA2G Buffer A	5.000
5X KAPA Enhancer 1	5.000
F Leo Primer (10µM) Leo006	0.500
R Leo Primer (10µM) Leo006	0.500
F Leo Primer (10µM) Leo045	0.500
R Leo Primer (10µM) Leo045	0.500
F Leo Primer (10µM) Leo126	0.500
R Leo Primer (10µM) Leo126	0.500
F Leo Primer (10µM) Leo281	0.750
R Leo Primer (10µM) Leo281	0.750
dNTPs (10mM ea)	0.500
KAPA2G Robust HotStart Polymerase (5 units/µL)	0.100
DNA (≤20ng/uL)	1.000
TOTAL	**25.000**

Cycling Profile:
KAPA2G Robust TD 30

Stage 1		
95	3m	x1
Stage 2-6		
95	15s	x1
60-56	15s	
72	15s	
Stage 7		
95	15s	x25
55	15s	
72	15s	
Stage 8		
72	1m	x1
4	∞	

KAPA2G Robus HotStart PCR Protocol - 25µL Rxn

updated: 10/17/2017 (CJC) - Mix 2	AMOUNT
ddH2O	7.400
5X KAPA2G Buffer A	5.000
5X KAPA Enhancer 1	5.000
F Leo Primer (10µM) Leo008	1.000
R Leo Primer (10µM) Leo008	1.000
F Leo Primer (10µM) Leo077	0.500
R Leo Primer (10µM) Leo077	0.500
F Leo Primer (10µM) Leo085	0.500
R Leo Primer (10µM) Leo085	0.500
F Leo Primer (10µM) Leo391	1.000
R Leo Primer (10µM) Leo391	1.000
dNTPs (10mM ea)	0.500
KAPA2G Robust HotStart Polymerase (5 units/µL)	0.100
DNA (≤20ng/uL)	1.000
TOTAL	**25.000**

Cycling Profile:
KAPA2G Robust TD 30

Stage 1		
95	3m	x1
Stage 2-6		
95	15s	x1
60-56	15s	
72	15s	
Stage 7		
95	15s	x25
55	15s	
72	15s	
Stage 8		
72	1m	x1
4	∞	

KAPA2G Robus HotStart PCR Protocol - 25µL Rxn

updated: 10/17/2017 (CJC) - Mix 3	AMOUNT
ddH2O	9.400
5X KAPA2G Buffer A	5.000
5X KAPA Enhancer 1	5.000
F Leo Primer (10µM) Leo224	0.500
R Leo Primer (10µM) Le0224	0.500
F Leo Primer (10µM) Leo247	0.500
R Leo Primer (10µM) Leo247	0.500
F Leo Primer (10µM) Leo031	1.000
R Leo Primer (10µM) Leo031	1.000
dNTPs (10mM ea)	0.500
KAPA2G Robust HotStart Polymerase (5 units/µL)	0.100
DNA (≤20ng/uL)	1.000
TOTAL	**25.000**

Cycling Profile:
KAPA2G Robust TD 30

Stage 1		
95	3m	x1
Stage 2-6		
95	15s	x1
60-56	15s	
72	15s	
Stage 7		
95	15s	x25
55	15s	
72	15s	
Stage 8		
72	1m	x1
4	∞	

KAPA2G Robus HotStart PCR Protocol - 25µL Rxn

updated: 3/29/2017 (CJC) - Leo230	AMOUNT
ddH2O	12.400
5X KAPA2G Buffer A	5.000
5X KAPA Enhancer 1	5.000
F Leo Primer (10µM) Leo230	0.500
R Leo Primer (10µM) Leo230	0.500
dNTPs (10mM ea)	0.500
KAPA2G Robust HotStart Polymerase (5 units/µL)	0.100
DNA (≤20ng/uL)	1.000
TOTAL	**25.000**

Cycling Profile:
KAPA2G Robust TD 35

Stage 1		
95	3m	x1
Stage 2-6		
95	15s	x1
60-56	15s	
72	15s	
Stage 7		
95	15s	x30
55	15s	
72	15s	
Stage 8		
72	1m	x1
4	∞	

KAPA2G Robus HotStart PCR Protocol - 25µL Rxn

updated: 3/29/2017 (CJC) - Leo506	AMOUNT
ddH2O	12.400
5X KAPA2G Buffer A	5.000
5X KAPA Enhancer 1	5.000
F Leo Primer (10µM) Leo506	0.500
R Leo Primer (10µM) Leo506	0.500
dNTPs (10mM ea)	0.500
KAPA2G Robust HotStart Polymerase (5 units/µL)	0.100
DNA (≤20ng/uL)	1.000
TOTAL	**25.000**

KAPA2G Robus HotStart PCR Protocol - 25µL Rxn

updated: 3/29/2017 (CJC) - Leo098	AMOUNT
ddH2O	12.400
5X KAPA2G Buffer A	5.000
5X KAPA Enhancer 1	5.000
F Leo Primer (10µM) Leo098	0.500
R Leo Primer (10µM) Leo098	0.500
dNTPs (10mM ea)	0.500
KAPA2G Robust HotStart Polymerase (5 units/µL)	0.100
DNA (≤20ng/uL)	1.000
TOTAL	**25.000**

Cycling Profile:
KAPA2G Robust TD 25

Stage 1		
95	3m	x1
Stage 2-6		
95	15s	x1
60-56	15s	
72	15s	
Stage 7		
95	15s	x20
55	15s	
72	15s	
Stage 8		
72	1m	x1
4	∞	

KAPA2G Robus HotStart PCR Protocol - 25µL Rxn

updated: 3/29/2017 (CJC) - IND STR	AMOUNT
ddH2O	12.400
5X KAPA2G Buffer A	5.000
5X KAPA Enhancer 1	5.000
F Leo Primer (10µM)	0.500
R Leo Primer (10µM)	0.500
dNTPs (10mM ea)	0.500
KAPA2G Robust HotStart Polymerase (5 units/µL)	0.100
DNA (≤20ng/uL)	1.000
TOTAL	**25.000**

Cycling Profile:
KAPA2G Robust TD 30

Stage 1		
95	3m	x1
Stage 2-6		
95	15s	x1
60-56	15s	
72	15s	
Stage 7		
95	15s	x25
55	15s	
72	15s	
Stage 8		
72	1m	x1
4	∞	

A.2.d: PCR Protocol for mtDNA Genes 12S-16S

KAPA2G Robus HotStart PCR Protocol - 25µL Reaction

updated: 08/21/2014 (CJC)	AMOUNT	
ddH2O	10.900	0.000
5X KAPA2G Buffer A	5.000	0.000
5X KAPA Enhancer 1	5.000	0.000
12S-UP-F Primer (10µM)	1.250	0.000
PAN-16S-R Primer (10µM)	1.250	0.000
dNTPs (10mM)	0.500	0.000
KAPA2G Robust HotStart Polymerase (5 units/µL)	0.100	0.000
DNA (≤20ng/uL)	1.000	
TOTAL	**25.000**	**0.000**

Cycling Profile: KAPA2G Robust

95	3m	x1
95	15s	x35
55*	30s	
72	60s	
72	1m	x1
4	∞	

*12S-UP-F Tm = 69; PAN-16S-R Tm = 49

A.2.e: BigDye Terminator & XTerminator Purification for Sequencing

Sequencing

Cleanup PCR product **:**

5 µL PCR product

2 µL ExoSap-IT

- Vortex & Spin

PCR profile: ExoSap-IT

37	15m	x1
85	15m	
4	∞	

BigDye *(Master Mix per Primer)*	**AMOUNT**	26.000
ddH2O	5.500	143.000
5X COLORLESS buffer	1.000	26.000
Primer	0.500	13.000
BigDye	2.000	52.000
Cleaned-up DNA	1.000	
TOTAL	**10.000**	**234.000**

PCR profile: BigDye_Kit_Std*

95	1m	x1
96	10s	x35
50	5s	
60	4m	
60	10m	x1
4	∞	

Capillary Electrophoresis for TAGC

Warm SAM solution in 37°C incubator to resuspend
Centrifuge samples
Add 4.5x volume (45µL) SAM solution to each well
Vortex x-terminator solution 10 sec - immediately pipette 1x volume (10µL) into each well
Vortex full speed for 30 min
Centrifuge @ 1000g for 2 min
Remove & use supernatant (discard solids)
Run on 3130 (or store at 4°C until ready)

APPENDIX B

SAMPLE LISTS & DATA

1. Sample Information

    a. Zambian Lions (8 Pages)
    b. Modern Lions – Nuclear Analysis (2 Pages)
    c. Modern Lions – Mitochondrial Analysis (1 Page)
    d. Historical Lions – Location Information (2 Pages)
    e. Historical Lions – Results (2 Pages)

2. STR Allele Calls

    a. Zambian Lions (11 Pages)
    b. Modern Population (4 Pages)
    c. Historical Population (5 Pages)

3. Mitochondrial Sequences

    a. Novel 12S to 16S (6)
    b. FASTAs of the 280 Mitogenomic Polymorphic Sites (89)

B.1.a: ZAMBIAN LIONS (8 PAGES)

Sample Information			Animal Information						mtDNA	STRs	*STRUCTURE*	
TAMUID	Type	Date	Sex	Area	Location	S	E	Source	Haplotype	# Loci	Western	Eastern
2011000254	Hide	2008	M	KF	25	14 18 76.2	26 50 22.6	GPS	H9	14	0.9971	0.0029
2011000255	Hide	2009	M	KF	25	14 20 45.1	26 43 87.1	GPS	H9	13	0.9879	0.0121
2011000256	Hide	2008	M	KF	25	14 20 45.1	26 43 87.1	GPS	H9	13	0.9971	0.0029
2011000257	Hide	2008	M	KF	25	14 16 79.7	26 44 17.3	GPS	H9	13	0.9919	0.0081
2011000258	Hide	2008	M	LV	3	11 02 44.2	32 52 26.3	Central	H11	14	0.0016	0.9984
2011000259	Hide	2008	M	LV	3	11 02 44.2	32 52 26.3	Central	Z1	14	0.0097	0.9903
2011000260	Hide	2008	M	LV	3	11 02 44.2	32 52 26.3	Central	Z1	14	0.0013	0.9987
2011000261	Hide	2008	M	LV	2	11 01 35.8	32 05 33.4	Central	Z1	14	0.0054	0.9946
2011000262	Hide	2008	M	LV	2	11 01 35.8	32 05 33.4	Central	Z1	14	0.2873	0.7127
2011000263	Hide	2008	M	LV	8	12 21 24.5	31 55 10.6	GPS	H11	14	0.0095	0.9905
2011000264	Hide	2008	M	LV	8	12 16 37.5	32 03 22.4	GPS	Z1	14	0.0032	0.9968
2011000265	Hide	2008	M	KF	38	14 33 27.2	26 37 45.2	Central	H9	13	0.9932	0.0068
2011000267	Hide	2008	M	KF	31	15 58 55.0	25 59 35.7	Central	H9	13	0.9959	0.0041
2011000268	Hide	2008	M	LV	14	14 03 35.9	31 13 45.1	Central	Z1	13	0.0044	0.9956
2011000269	Hide	2008	M	LV	14	14 03 35.9	31 13 45.1	Central	H11	14	0.0033	0.9967
2011000270	Hide	2008	M	CO	40	14 05 46.7	30 43 42.5	Central	Z1	13	0.0691	0.9309
2011000271	Hide	2008	M	CO	40	14 05 46.7	30 43 42.5	Central	H11	14	0.0649	0.9351
2011000272	Hide	2008	M	CO	15	13 46 32.4	30 43 53.8	Central		14	0.0547	0.9453
2011000273	Hide	2008	M	LV	5	11 57 98.0	32 24 93.0	GPS	H11	13	0.0029	0.9971
2011000274	Hide	2008	M	LV	5	11 46 41.1	32 32 77.8	GPS	Z1	13	0.0017	0.9983
2011000275	Hide	2008	M	LV	7	12 26 07.2	32 21 51.4	Central	Z1	14	0.0015	0.9985
2011000276	Hide	2008	M	LV	7	12 26 07.2	32 21 51.4	Central	Z1	14	0.0033	0.9967
2011000277	Hide	2008	M	LV	7	12 26 07.2	32 21 51.4	Central	Z1	14	0.0043	0.9957
2011000278	Hide	2008	M	LV	6	12 17 04.5	32 28 46.5	Central	Z1	14	0.0021	0.9979
2011000279	Hide	2008	M	LV	6	12 17 04.5	32 28 46.5	Central	Z1	13	0.0065	0.9935
2011000280	Hide	2008	M	LV	6	12 17 04.5	32 28 46.5	Central	Z1	13	0.0042	0.9958
2011000281	Hide	2008	M	CO	42	14 12 23.8	30 35 38.5	GPS	Z1	13	0.094	0.906
2011000282	Hide	1984	M	LV	5	11 50 50.5	32 20 20.3	Central		14	0.0021	0.9979
2011000283	Hide	2008	F	CO	17	14 17 44.9	30 32 56.4	GPS	H11	13	0.0301	0.9699
2011000284	Hide	2008	F	CO	17	14 17 44.9	30 32 56.4	GPS	H11	13	0.0372	0.9628
2011000285	Hide	2008	F	LV	C	13 10 00.4	31 42 36.5	GPS	Z1	14	0.0043	0.9957
2011000286	Hide	2008	F	LV	B	12 53 55.0	31 45 06.4	GPS	Z1	14	0.0026	0.9974
2011000287	Hide	2008	M	OUT	OUT	14 54 57.4	28 25 13.7	Central	H9	14	0.414	0.586
2011000288	Hide	2008	M	KF	23	13 24 42.2	31 32 38.5	GPS	H9	14	0.9967	0.0033
2011000290	Hide	2004	M	CO	17	14 18 43.9	30 21 56.9	Central	Z1	13	0.0415	0.9585
2011000291	Hide	2005	M	CO	17	14 18 43.9	30 21 56.9	Central	Z1	14	0.0388	0.9612
2011000293	Bone	2005	M	KF	25	13 55 48.6	26 35 46.2	Central	H9	0	na	na
2011000295	Hide	2006	M	LV	8	12 21 59.0	31 56 42.0	GPS	H11	13	0.0065	0.9935
2011000296	Hide	2006	M	LV	8	12 15 81.2	32 09 93.8	GPS		14	0.0053	0.9947
2011000297	Hide	2006	U	LV	8	12 09 10.6	31 50 49.8	Central	H11	14	0.0054	0.9946
2011000298	Hide	2006	M	LV	10	12 44 65.4	32 03 64.4	GPS	Z1	13	0.0015	0.9985
2011000299	Hide	2006	M	LV	10	12 42 19.3	32 15 22.2	Central	Z1	13	0.0017	0.9983
2011000300	Hide	2006	M	LV	7	12 26 07.2	32 21 51.4	Central	Z1	14	0.0024	0.9976
2011000301	Hide	2006	M	LV	7	12 26 07.2	32 21 51.4	Central	Z1	14	0.0015	0.9985
2011000302	Hide	2006	M	LV	6	12 17 04.5	32 28 46.5	Central		14	0.0017	0.9983
2011000303	Hide	2006	M	LV	6	12 38 49.0	32 18 53.7	GPS	Z1	14	0.0032	0.9968
2011000304	Hide	2006	M	LV	6	12 17 04.5	32 28 46.5	Central	Z6	14	0.0027	0.9973
2011000305	Hide	2006	M	LV	6	12 17 04.5	32 28 46.5	Central	H11	14	0.0033	0.9967
2011000306	Hide	2006	M	CO	17	14 18 43.9	30 21 56.9	Central		14	0.0541	0.9459
2011000307	Hide	2006	M	CO	17	14 18 43.9	30 21 56.9	Central	H11	14	0.0203	0.9797
2011000308	Hide	2006	M	KF	25	16 27 16.9	26 34 55.6	GPS	Z3	14	0.9959	0.0041
2011000309	Bone	2006	M	KF	31	15 58 55.0	25 59 35.7	Central		13	0.9967	0.0033
2011000310	Hide	2006	M	KF	38	14 33 27.2	26 37 45.2	Central	Z4	14	0.9977	0.0023
2011000311	Hide	2006	M	LV	12	13 28 21.4	31 52 20.9	Central	Z1	13	0.0018	0.9982
2011000312	Hide	2006	M	LV	9	12 31 39.0	32 03 93.0	GPS	Z1	14	0.0013	0.9987
2011000313	Hide	2006	M	CO	44	14 37 01.0	30 25 39.0	GPS	Z1	14	0.3323	0.6677

B.1.a: ZAMBIAN LIONS (PAGE 2 OF 8)

Sample Information			Animal Information						mtDNA	STRs	*STRUCTURE*	
TAMUID	Type	Date	Sex	Area	Location	S	E	Source	Haplotype	# Loci	Western	Eastern
2011000314	Hide	2006	F	CO	44	14 37 00.0	30 25 00.0	GPS	H11	14	0.0441	0.9559
2011000315	Hide	2006	M	CO	42	14 12 31.7	30 36 34.6	Central	H11	14	0.0243	0.9757
2011000316	Hide	2006	M	CO	42	14 12 31.7	30 36 34.6	Central	Z1	13	0.1375	0.8625
2011000317	Hide	2006	M	LV	5	11 46 27.9	32 31 99.7	GPS	Z1	13	0.0054	0.9946
2011000318	Hide	2006	M	LV	5	12 06 63.2	32 17 46.9	GPS	H11	14	0.0018	0.9982
2011000319	Hide	2006	M	LV	5	11 46 27.9	32 31 99.7	GPS	Z1	13	0.0183	0.9817
2011000320	Hide	2006	M	LV	5	11 49 93.2	32 27 63.8	GPS	Z1	14	0.0021	0.9979
2011000321	Hide	2006	M	LV	5	12 06 95.1	32 17 19.1	GPS	H11	14	0.0031	0.9969
2011000322	Hide	2006	M	LV	3	11 02 44.2	32 52 26.3	Central	H11	14	0.0016	0.9984
2011000323	Hide	2006	M	LV	3	11 02 44.2	32 52 26.3	Central	H11	14	0.0029	0.9971
2011000324	Hide	2006	M	LV	3	11 02 44.2	32 52 26.3	Central	Z1	14	0.0026	0.9974
2011000325	Hide	2006	M	KF	23	14 04 47.3	25 43 38.1	GPS	Z3	14	0.9966	0.0034
2011000326	Hide	2006	M	KF	23	14 05 21.6	25 47 64.8	GPS	Z3	14	0.9878	0.0122
2011000327	Hide	2006	M	KF	23	14 05 30.6	25 40 75.3	GPS	H9	14	0.9974	0.0026
2011000328	Hide	2006	M	KF	24	14 04 31.5	26 15 12.6	Central	H9	14	0.9915	0.0085
2011000329	Hide	2006	M	KF	24	14 04 31.5	26 15 12.6	Central	H9	14	0.9976	0.0024
2011000330	Hide	2007	M	LV	8	12 23 62.2	32 07 23.1	GPS	Z1	14	0.0053	0.9947
2011000331	Hide	2007	M	LV	8	12 26 01.0	32 03 36.0	GPS	H11	14	0.0024	0.9976
2011000332	Hide	2007	M	LV	8	12 25 33.2	32 03 00.0	GPS	H11	14	0.0053	0.9947
2011000333	Hide	2007	M	LV	12	13 31 11.0	31 50 00.0	GPS	Z1	14	0.0082	0.9918
2011000334	Hide	2007	M	LV	12	13 31 11.0	31 50 00.0	GPS	Z1	13	0.0021	0.9979
2011000335	Hide	2007	F	LV	B	12 52 11.9	32 03 29.4	GPS	Z1	13	0.0023	0.9977
2011000336	Hide	2007	F	LV		12 52 11.9	32 03 29.4	Central		14	0.0015	0.9985
2011000337	Hide	2007	M	LV	10	12 42 19.3	32 15 22.2	Central	Z1	14	0.004	0.996
2011000338	Hide	2007	M	LV	10	12 42 19.3	32 15 22.2	Central		14	0.003	0.997
2011000339	Hide	2007	M	LV	10	12 42 19.3	32 15 22.2	Central		14	0.0023	0.9977
2011000340	Hide	2007	M	KF	35	16 23 58.5	25 26 49.7	Central	H9	13	0.9891	0.0109
2011000341	Hide	2007	M	KF	30	15 20 32.4	26 05 17.6	Central	H9	13	0.9911	0.0089
2011000342	Hide	2007	M	CO	40	14 05 46.7	30 43 42.5	Central	Z1	13	0.0305	0.9695
2011000343	Hide	2007	M	KF	38	14 33 27.2	26 37 45.2	Central	H9	12	0.9961	0.0039
2011000344	Hide	2007	M	KF	38	14 33 27.2	26 37 45.2	Central	H9	14	0.9909	0.0091
2011000346	Hide	2007	M	LV	2	11 01 35.8	32 05 33.4	Central	Z1	13	0.0033	0.9967
2011000347	Hide	2007	M	LV	3	11 02 44.2	32 52 26.3	Central		14	0.0011	0.9989
2011000348	Hide	2007	M	LV	3	11 02 44.2	32 52 26.3	Central	H11	13	0.0023	0.9977
2011000349	Hide	2007	M	LV	1	11 26 22.9	31 59 31.2	Central	Z1	14	0.0017	0.9983
2011000350	Hide	2007	M	LV	11	12 58 12.1	32 03 37.1	Central		13	0.0017	0.9983
2011000351	Hide	2007	M	LV	11	13 00 37.0	32 04 37.6	GPS	Z1	13	0.0032	0.9968
2011000352	Hide	2007	M	LV	11	13 01 76.1	31 54 72.1	GPS	Z1	14	0.0013	0.9987
2011000353	Hide	2007	M	LV	13	13 17 52.2	31 44 18.1	Central	Z1	14	0.0041	0.9959
2011000354	Hide	2007	M	LV	13	13 16 41.8	31 39 87.1	GPS		14	0.0082	0.9918
2011000355	Hide	2007	M	ZA	20	15 29 82.7	30 14 07.0	GPS	Z2	14	0.1329	0.8671
2011000356	Hide	2007	M	KF	25	13 55 48.6	26 35 46.2	Central	H9	14	0.9912	0.0088
2011000357	Hide	2007	M	KF	24	14 04 31.5	26 15 12.6	Central	H9	13	0.9928	0.0072
2011000358	Hide	2007	M	KF	23	14 15 43.6	25 32 15.4	GPS	H9	13	0.9963	0.0037
2011000359	Hide	2007	M	KF	23	14 09 07.8	25 33 17.5	GPS	Z3	14	0.996	0.004
2011000360	Hide	2007	M	KF	23	14 09 07.8	25 33 17.5	GPS	Z3	12	0.9907	0.0093
2011000361	Hide	2007	M	LV	5	11 47 53.6	32 30 38.0	GPS	Z1	14	0.0017	0.9983
2011000362	Hide	2007	M	LV	5	11 56 40.8	32 24 86.7	GPS	H11	14	0.0027	0.9973
2011000363	Hide	2007	M	LV	5	12 07 23.1	32 17 45.0	GPS	Z1	14	0.0033	0.9967
2011000364	Hide	2007	M	LV	5	11 57 68.8	32 24 62.7	GPS	H11	14	0.0017	0.9983
2011000365	Hide	2007	M	LV	6	12 17 04.5	32 28 46.5	Central	Z1	14	0.0021	0.9979
2011000366	Hide	2007	M	LV	6	12 17 04.5	32 28 46.5	Central	H11	14	0.0059	0.9941
2011000367	Hide	2007	M	LV	6	12 17 04.5	32 28 46.5	Central	H11	14	0.0023	0.9977
2011000368	Hide	2007	M	LV	6	12 17 04.5	32 28 46.5	Central	Z1	14	0.0047	0.9953
2011000369	Hide	2007	M	LV	7	12 26 07.2	32 21 51.4	Central	Z1	14	0.0016	0.9984
2011000370	Hide	2007	M	LV	7	12 26 07.2	32 21 51.4	Central	Z1	14	0.1086	0.8914

B.1.a: ZAMBIAN LIONS (PAGE 3 OF 8)

Sample Information			Animal Information						mtDNA	STRs	*STRUCTURE*	
TAMUID	Type	Date	Sex	Area	Location	S	E	Source	Haplotype	# Loci	Western	Eastern
2011000371	Hide	2007	M	LV	7	12 26 07.2	32 21 51.4	Central	H11	14	0.0072	0.9928
2011000372	Hide	2007	M	CO	42	14 12 31.7	30 36 34.6	Central	H11	14	0.0287	0.9713
2011000373	Hide	2007	M	CO	42	14 12 31.7	30 36 34.6	Central		12	0.0274	0.9726
2011000376	Hide	2011	M	LV	8	12 09 10.6	31 50 49.8	Central	Z1	13	0.0033	0.9967
2011000377	Hide	2011	M	LV	6	12 17 04.5	32 28 46.5	Central	Z1	14	0.0043	0.9957
2011000378	Hide	2011	M	LV	6	12 17 04.5	32 28 46.5	Central	Z1	14	0.0033	0.9967
2011000379	Hide	2011	M	LV	7	12 26 07.2	32 21 51.4	Central	H11	13	0.0025	0.9975
2011000380	Hide	2011	M	LV	9	12 33 35.9	32 01 40.6	GPS	Z1	14	0.0046	0.9954
2011000383	Hide	2011	M	KF	31	15 58 55.0	25 59 35.7	Central	Z3	14	0.9928	0.0072
2011000385	Hide	2011	M	KF	28	15 09 24.3	26 09 48.8	Central	H9	14	0.9933	0.0067
2011000387	Hide	2011	M	CO	40	14 05 46.7	30 43 42.5	Central	H11	14	0.1196	0.8804
2011000388	Hide	2011	M	LV	5	11 50 50.5	32 20 20.3	Central	Z1	14	0.0015	0.9985
2011000389	Hide	2011	M	KF	28	15 09 24.3	26 09 48.8	Central	Z4	14	0.9777	0.0223
2011000390	Hide	2011	M	LV	6	12 17 04.5	32 28 46.5	Central	H11	13	0.0054	0.9946
2011000391	Hide	2011	M	KF	25	13 55 48.6	26 35 46.2	Central	Z4	14	0.9913	0.0087
2011000392	Hide	2011	M	KF	25	13 55 48.6	26 35 46.2	Central	Z3	13	0.9973	0.0027
2011000393	Hide	2011	M	CO	42	14 12 31.7	30 36 34.6	Central	H11	14	0.1011	0.8989
2011000394	Hide	2011	M	KF	35	16 23 58.5	25 26 49.7	Central	H9	13	0.9951	0.0049
2011000395	Hide	2011	M	KF	35	16 19 22.2	25 31 07.7	GPS	Z5	13	0.997	0.003
2011000396	Hide	2011	M	KF	25	13 55 48.6	26 35 46.2	Central	H9	14	0.998	0.002
2011000397	Hide	2011	M	KF	38	14 33 27.2	26 37 45.2	Central	Z3	14	0.9959	0.0041
2011000398	Hide	2011	M	CO	41	14 37 17.7	30 25 61.3	Central	H11	14	0.0275	0.9725
2011000399	Hide	2011	M	LV	5	11 50 50.5	32 20 20.3	Central	H11	14	0.0026	0.9974
2011000400	Hide	2011	M	LV	11	13 04 52.7	31 49 51.2	GPS	Z1	14	0.0027	0.9973
2011000401	Hide	2011	M	KF	38	14 33 27.2	26 37 45.2	Central	H9	14	0.9843	0.0157
2011000403	Hide	2011	M	LV	9	12 33 35.9	32 01 40.6	GPS	H9	14	0.0015	0.9985
2011000405	Hide	2011	M	LV	8	12 09 10.6	31 50 49.8	Central	Z1	13	0.0677	0.9323
2011000406	Hide	2011	M	KF	28	15 10 54.9	26 29 00.4	Central	H9	14	0.9981	0.0019
2011000408	Hide	2011	M	LV	5	11 50 50.5	32 20 20.3	Central	H11	14	0.0023	0.9977
2011000409	Hide	2011	M	KF	28	15 09 24.3	26 09 48.8	Central	Z4	14	0.9917	0.0083
2011000410	Bone	2009	M	SI	OUT	11 02 44.2	32 52 26.3	Central	H11	0	na	na
2011000411	Hide	2011	M	CO	17	14 24 21.6	30 27 29.3	GPS	H11	14	0.0316	0.9684
2011000412	Hide	2011	M	CO	17	14 13 49.9	30 34 27.7	GPS	Z1	14	0.0252	0.9748
2011000413	Hide	2009	M	KF	28	15 09 24.3	26 09 48.8	Central	H9	13	0.9967	0.0033
2011000414	Hide	2009	M	LV	3	11 02 44.2	32 52 26.3	Central	H11	14	0.0017	0.9983
2011000415	Hide	2009	M	LV	5	11 50 50.5	32 20 20.3	Central	Z1	14	0.0036	0.9964
2011000416	Hide	2009	F	KF	F	15 02 34.2	25 59 57.3	Central	Z5	0	na	na
2011000417	Hide	2009	F	LV	B	13 04 42.9	31 47 25.7	Central	Z1	14	0.0019	0.9981
2011000418	Hide	2009	F	LV	B	13 04 42.9	31 47 25.7	Central	Z1	14	0.0017	0.9983
2011000420	Hide	2009	F	LV	B	13 04 42.9	31 47 25.7	Central	Z1	14	0.0018	0.9982
2011000421	Hide	2009	M	LV	B	13 04 42.9	31 47 25.7	Central		10	0.012	0.988
2011000422	Hide	2009	F	ZA	D	15 49 16.5	29 04 17.3	Central	Z1	14	0.0914	0.9086
2011000424	Hide	2009	M	LV	6	12 17 04.5	32 28 46.5	Central	Z1	14	0.0056	0.9944
2011000425	Hide	2009	M	LV	3	11 02 44.2	32 52 26.3	Central	Z1	14	0.0025	0.9975
2011000426	Hide	2009	M	KF	E	14 54 17.6	25 28 09.7	GPS	H9	14	0.9933	0.0067
2011000427	Hide	2009	F	LV	B	13 04 42.9	31 47 25.7	Central	Z1	14	0.0017	0.9983
2011000428	Hide	2009	M	LV	6	12 17 04.5	32 28 46.5	Central	Z1	14	0.002	0.998
2011000429	Hide	2009	M	KF	38	14 28 07.2	26 35 16.6	GPS	H9	14	0.9969	0.0031
2011000430	Hide	2009	M	LV	6	12 17 04.5	32 28 46.5	Central	H11	14	0.0055	0.9945
2011000431	Hide	2009	M	LV	3	11 02 44.2	32 52 26.3	Central	Z1	14	0.0034	0.9966
2011000432	Hide	2009	M	KF	25	13 55 48.6	26 35 46.2	Central	Z1	14	0.1939	0.8061
2011000433	Hide	2009	M	LV	2	11 01 35.8	32 05 33.4	Central	Z1	14	0.0018	0.9982
2011000434	Hide	2009	M	KF	E	14 54 17.6	25 28 09.7	GPS	H9	14	0.9961	0.0039
2011000435	Hide	2009	M	LV	7	12 26 07.2	32 21 51.4	Central	Z1	14	0.0031	0.9969
2011000436	Hide	2009	M	LV	8	12 16 22.0	32 03 13.0	GPS	Z1	14	0.0045	0.9955
2011000437	Hide	2009	M	LV	10	12 42 19.3	32 15 22.2	Central	Z1	14	0.0024	0.9976

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Sample Information			Animal Information						mtDNA	STRs	*STRUCTURE*	
TAMUID	Type	Date	Sex	Area	Location	S	E	Source	Haplotype	# Loci	Western	Eastern
2011000438	Hide	2009	M	KF	25	13 55 48.6	26 35 46.2	Central	H9	14	0.9937	0.0063
2011000440	Hide	2009	M	SI	OUT	17 30 45.6	23 31 32.0	Central	H9	11	0.9037	0.0963
2011000441	Hide	2009	M	LV	7	12 26 07.2	32 21 51.4	Central	Z1	14	0.0013	0.9987
2011000442	Hide	2009	M	LV	7	12 26 07.2	32 21 51.4	Central	Z1	14	0.0033	0.9967
2011000443	Hide	2009	M	KF	35	16 23 58.5	25 26 49.7	Central	H9	14	0.9934	0.0066
2011000444	Hide	2009	M	LV	13	13 17 52.2	31 44 18.1	Central	Z1	14	0.005	0.995
2011000446	Hide	2009	F	ZA	D	15 49 16.5	29 04 17.3	Central	H11	14	0.1446	0.8554
2011000447	Hide	2009	M	KF	25	13 55 48.6	26 35 46.2	Central	H9	14	0.9953	0.0047
2011000448	Hide	2009	M	LV	8	12 09 10.6	31 50 49.8	Central	Z6	14	0.0433	0.9567
2011000449	Hide	2009	M	CO	40	14 05 46.7	30 43 42.5	Central	H11	14	0.0329	0.9671
2011000450	Hide	2009	M	KF	25	13 55 48.6	26 35 46.2	Central	H9	14	0.9909	0.0091
2011000452	Hide	2009	M	KF	24	14 04 31.5	26 15 12.6	Central	H9	14	0.994	0.006
2011000453	Hide	2009	M	LV	5	11 50 50.5	32 20 20.3	Central	Z1	14	0.0027	0.9973
2011000454	Hide	2009	M	KF	24	14 04 31.5	26 15 12.6	Central	H9	14	0.9951	0.0049
2011000455	Hide	2009	M	LV	10	12 42 19.3	32 15 22.2	Central	Z1	14	0.0015	0.9985
2011000456	Hide	2009	M	LV	5	11 50 50.5	32 20 20.3	Central	Z1	14	0.0018	0.9982
2011000457	Hide	2009	M	KF	35	16 23 58.5	25 26 49.7	Central	H9	14	0.9438	0.0562
2011000458	Hide	2009	M	CO	42	14 12 31.7	30 36 34.6	Central	Z1	14	0.04	0.96
2011000459	Hide	2009	M	CO	40	14 05 46.7	30 43 42.5	Central	Z6	14	0.0295	0.9705
2011000460	Hide	2008	M	OUT	OUT	14 54 57.4	28 25 13.7	Central	H11	14	0.2861	0.7139
2011000461	Hide	2009	M	KF	F	14 19 06.7	25 57 54.1	Central	H9	14	0.9963	0.0037
2011000462	Hide	2009	F	KF	F	14 19 06.7	25 57 54.1	Central	Z3	14	0.9909	0.0091
2011000463	Hide	2010	M	KF	35	16 23 58.5	25 26 49.7	Central	H9	14	0.9947	0.0053
2011000465	Hide	2010	M	LV	10	12 42 19.3	32 15 22.2	Central	Z1	14	0.0034	0.9966
2011000466	Hide	2010	M	LV	8	12 20 33.4	32 11 83.6	GPS	Z1	14	0.09	0.91
2011000467	Hide	2010	M	LV	8	12 21 70.9	31 54 69.8	GPS	Z1	14	0.0015	0.9985
2011000468	Hide	2010	M	LV	7	12 26 07.2	32 21 51.4	Central	Z1	14	0.0018	0.9982
2011000470	Hide	2010	M	KF	28	15 09 24.3	26 09 48.8	Central	H9	14	0.9951	0.0049
2011000471	Hide	2010	M	LV	7	12 26 07.2	32 21 51.4	Central	H11	14	0.0014	0.9986
2011000472	Hide	2010	M	LV	2	11 01 35.8	32 05 33.4	Central	H11	14	0.0015	0.9985
2011000473	Hide	2010	M	LV	10	12 42 19.3	32 15 22.2	Central	Z1	14	0.007	0.993
2011000474	Hide	2010	M	KF	25	13 55 48.6	26 35 46.2	Central	H9	13	0.9926	0.0074
2011000475	Hide	2010	M	LV	7	12 26 07.2	32 21 51.4	Central	H11	13	0.0047	0.9953
2011000476	Hide	2010	M	KF	28	15 09 24.3	26 09 48.8	Central	H9	14	0.9964	0.0036
2011000477	Hide	2010	M	KF	24	14 04 31.5	26 15 12.6	Central	H9	14	0.9967	0.0033
2011000478	Hide	2010	M	LV	10	12 42 19.3	32 15 22.2	Central	Z1	14	0.0059	0.9941
2011000480	Hide	2010	M	CO	40	14 05 46.7	30 43 42.5	Central	Z1	14	0.0363	0.9637
2011000481	Hide	2010	M	KF	25	13 55 48.6	26 35 46.2	Central	H9	14	0.9973	0.0027
2011000482	Hide	2010	M	KF	24	14 04 31.5	26 15 12.6	Central	H9	14	0.9953	0.0047
2011000483	Hide	2010	M	LV	6	12 17 04.5	32 28 46.5	Central	H11	14	0.0019	0.9981
2011000484	Hide	2010	F	KF	F	15 54 08.7	25 57 37.4	GPS	Z3	14	0.9959	0.0041
2011000485	Hide	2010	M	LV	5	11 50 50.5	32 20 20.3	Central	Z1	14	0.0013	0.9987
2011000486	Hide	2010	M	KF	25	13 55 48.6	26 35 46.2	Central	Z3	14	0.9968	0.0032
2011000487	Hide	2010	M	KF	23	14 19 43.2	25 30 90.4	GPS	H9	14	0.9967	0.0033
2011000488	Hide	2010	M	KF	31	05 47 49.6	26 04 06.9	GPS	Z3	12	0.9871	0.0129
2011000489	Hide	2010	M	KF	35	16 21 46.2	25 29 50.2	GPS	H9	14	0.9893	0.0107
2011000491	Hide	2010	M	LV	3	11 02 44.2	32 52 26.3	Central	H11	14	0.0014	0.9986
2011000492	Hide	2010	M	LV	6	12 17 04.5	32 28 46.5	Central	Z1	14	0.0011	0.9989
2011000493	Hide	2010	M	KF	23	14 03 77.6	25 48 37.0	GPS	H9	14	0.9973	0.0027
2011000494	Hide	2010	F	KF	F	16 09 13.9	26 01 43.4	GPS		13	0.9959	0.0041
2011000495	Hide	2010	M	LV	5	11 50 50.5	32 20 20.3	Central	Z1	14	0.0018	0.9982
2011000496	Hide	2010	M	KF	35	16 23 58.5	25 26 49.7	Central	H9	14	0.9978	0.0022
2011000497	Hide	2010	M	LV	7	12 26 07.2	32 21 51.4	Central	H11	14	0.0031	0.9969
2011000498	Hide	2010	M	LV	5	11 50 50.5	32 20 20.3	Central	H11	14	0.0023	0.9977
2011000499	Hide	2010	M	CO	17	14 18 43.9	30 21 56.9	Central	H11	14	0.0397	0.9603
2011000500	Hide	2010	M	LV	3	11 02 44.2	32 52 26.3	Central	Z1	14	0.0863	0.9137

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Sample Information			Animal Information						mtDNA	STRs	*STRUCTURE*	
TAMUID	Type	Date	Sex	Area	Location	S	E	Source	Haplotype	# Loci	Western	Eastern
2011000501	Hide	2010	M	KF	29	15 09 24.3	26 09 48.8	Central	H9	14	0.9975	0.0025
2011000502	Hide	2010	M	CO	42	14 12 31.7	30 36 34.6	Central	H11	14	0.0452	0.9548
2011000503	Hide	2010	M	LV	6	12 17 04.5	32 28 46.5	Central	Z1	14	0.0022	0.9978
2011000684	Biopsy	2008	M	KF	38	14 28 41.0	26 36 59.5	GPS	H9	14	0.9934	0.0066
2011000685	Biopsy	2008	M	KF	38	14 28 41.0	26 36 59.5	GPS	H9	14	0.9935	0.0065
2011000686	Biopsy	2008	M	LV	12	13 35 31.1	31 53 42.8	GPS	H11	14	0.0058	0.9942
2011000687	Biopsy	2008	F	LV	12	13 35 31.1	31 53 42.8	GPS	H11	14	0.0107	0.9893
2011000688	Biopsy	2008	M	LV		13 24 42.2	31 32 38.5	GPS	Z1	14	0.0052	0.9948
2011000690	Biopsy	2008	F	LV		13 17 48.3	31 38 34.1	GPS	Z1	13	0.0029	0.9971
2011000691	Biopsy	2008	F	LV	B	13 03 41.8	31 48 59.2	GPS	Z1	14	0.0023	0.9977
2011000692	Biopsy	2008	F	LV	C	13 10 19.8	31 42 06.2	GPS	Z1	14	0.0025	0.9975
2011000693	Biopsy	2008	F	LV	B	12 58 37.2	31 53 17.3	GPS	Z1	14	0.0037	0.9963
2011000694	Biopsy	2008	F	LV	A	11 55 28.1	32 15 22.4	GPS	H11	14	0.0017	0.9983
2011000695	Biopsy	2008	F	LV	A	11 56 16.5	32 16 38.1	GPS	Z1	14	0.0013	0.9987
2011000696	Biopsy	2008	F	LV	A	11 58 55.0	32 19 36.9	GPS	H11	14	0.0019	0.9981
2011000698	Biopsy	2008	M	LV	A	11 58 55.0	32 19 36.9	GPS		13	0.0017	0.9983
2011000699	Biopsy	2008	F	LV	A	11 55 33.5	32 15 08.2	GPS	H11	14	0.0015	0.9985
2011000703	Biopsy	2008	F	LV	A	11 55 17.4	32 24 41.1	GPS	H11	14	0.0027	0.9973
2011000704	Biopsy	2008	F	LV	A	11 55 17.4	32 24 41.0	GPS	H11	14	0.0161	0.9839
2011000705	Biopsy	2008	M	LV	A	11 55 17.4	32 24 41.0	GPS	H11	14	0.0027	0.9973
2011000706	Biopsy	2008	F	LV	6	12 28 17.0	32 11 32.8	GPS	Z1	14	0.0028	0.9972
2011000707	Biopsy	2008	F	LV	6	12 28 17.0	32 11 32.8	GPS	Z1	14	0.0095	0.9905
2011000708	Biopsy	2008	M	LV	B	12 56 54.8	31 45 42.1	GPS	Z1	14	0.0099	0.9901
2011000709	Biopsy	2008	M	LV	B	12 56 54.7	31 45 42.2	GPS	Z1	14	0.0038	0.9962
2011000710	Biopsy	2008	F	LV	B	12 56 54.7	31 45 42.2	GPS	Z1	14	0.0101	0.9899
2011000711	Biopsy	2008	F	LV	B	12 56 40.7	32 01 56.7	GPS	Z1	14	0.0087	0.9913
2011000712	Biopsy	2008	M	LV	B	12 56 40.7	32 01 56.7	GPS	Z1	14	0.0031	0.9969
2011000713	Biopsy	2008	F	LV	B	12 54 31.7	31 57 53.2	GPS	Z1	14	0.0035	0.9965
2011000714	Biopsy	2008	F	LV	B	12 54 31.7	31 57 53.2	GPS	Z1	14	0.0034	0.9966
2011000715	Biopsy	2008	M	LV	B	12 54 31.7	31 57 53.2	GPS	Z1	14	0.0015	0.9985
2011000716	Biopsy	2008	M	LV	B	12 54 31.7	31 57 53.2	GPS	Z1	14	0.0059	0.9941
2011000717	Biopsy	2008	F	LV	B	12 54 40.9	31 57 38.3	GPS	Z1	14	0.0018	0.9982
2011000718	Biopsy	2008	M	LV	B	12 54 40.6	31 57 38.3	GPS	Z1	14	0.0075	0.9925
2011000719	Biopsy	2008	M	LV	B	12 54 40.6	31 57 38.3	GPS	Z1	14	0.0015	0.9985
2011000720	Biopsy	2008	F	LV	B	12 54 34.1	31 57 41.1	GPS	Z1	14	0.0011	0.9989
2011000723	Biopsy	2008	F	LV	B	12 59 28.5	31 53 26.8	GPS	Z1	14	0.0037	0.9963
2011000724	Biopsy	2008	M	LV	B	12 59 28.4	31 53 26.8	GPS	Z1	14	0.0067	0.9933
2011000725	Biopsy	2008	M	LV	B	12 59 28.5	31 53 26.9	GPS	Z1	14	0.0057	0.9943
2011000727	Biopsy	2008	F	LV	13	13 12 53.8	31 41 35.5	GPS		12	0.0023	0.9977
2011000729	Biopsy	2008	F	KF	38	14 31 22.3	26 42 09.1	GPS	H9	14	0.9967	0.0033
2011000730	Biopsy	2008	F	KF	38	14 31 22.2	26 42 09.1	GPS	H9	0	na	na
2011000732	Biopsy	2008	F	KF	38	14 28 11.9	26 35 55.9	GPS	H9	0	na	na
2011000733	Biopsy	2008	F	KF	38	14 29 00.1	26 41 25.7	GPS	H9	14	0.997	0.003
2011000735	Biopsy	2005	M	LV	A	11 48 45.6	32 24 77.9	GPS	Z1	14	0.0018	0.9982
2011000736	Hide	2008	M	KF	23	14 15 06.0	25 33 19.0	GPS	H9	14	0.9967	0.0033
2011000737	Hide	2008	F	OUT	OUT	16 37 22.6	25 10 12.6	Central	H9	14	0.4199	0.5801
2011000738	Biopsy	2009	M	KF	25	14 26 50.2	26 35 02.6	GPS	H9	14	0.9951	0.0049
2011000739	Biopsy	2009	F	KF	25	14 26 50.2	26 35 02.5	GPS	H9	14	0.9951	0.0049
2011000740	Biopsy	2009	F	KF	25	14 26 50.1	26 35 02.6	GPS	H9	14	0.9944	0.0056
2011000741	Biopsy	2009	F	KF	38	14 28 14.9	26 35 24.1	GPS	H9	0	na	na
2011000742	Biopsy	2009	F	KF	38	14 28 14.8	26 35 24.1	GPS	H9	14	0.9933	0.0067
2011000743	Biopsy	2009	F	KF	38	14 34 32.5	26 42 01.1	GPS	H9	14	0.9967	0.0033
2011000744	Biopsy	2009	F	KF	38	14 34 32.6	26 42 00.9	GPS	H9	14	0.9975	0.0025
2011000745	Biopsy	2009	F	KF	38	14 28 02.0	26 34 51.1	GPS	H9	14	0.9973	0.0027
2011000746	Biopsy	2009	F	KF	28	15 14 45.1	25 59 17.1	GPS	H9	14	0.9159	0.0841
2011000748	Biopsy	2009	F	KF	31	16 05 52.4	26 02 42.5	Central	Z3	14	0.9958	0.0042

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Sample Information			Animal Information						mtDNA	STRs	*STRUCTURE*	
TAMUID	Type	Date	Sex	Area	Location	S	E	Source	Haplotype	# Loci	Western	Eastern
2011000749	Biopsy	2009	M	KF	31	15 49 27.3	26 00 39.0	GPS	Z3	14	0.9975	0.0025
2011000750	Biopsy	2009	M	KF	31	15 49 27.3	26 00 39.1	GPS	Z3	14	0.9881	0.0119
2011000751	Biopsy	2009	M	KF	31	15 49 27.3	26 00 39.1	GPS	Z3	14	0.9873	0.0127
2011000752	Biopsy	2009	M	KF	31	15 49 27.3	26 00 39.1	GPS	Z3	14	0.9966	0.0034
2011000753	Biopsy	2009	M	KF	F	15 58 02.0	25 55 38.8	GPS	H9	14	0.9917	0.0083
2011000754	Biopsy	2009	M	KF	F	15 58 02.0	25 55 38.8	GPS	Z3	14	0.9972	0.0028
2011000757	Biopsy	2009	M	KF	35	16 14 48.1	25 37 54.9	GPS	H9	14	0.9943	0.0057
2011000759	Hide	2009	M	KF	28	15 09 24.3	26 09 48.8	Central	H9	14	0.9963	0.0037
2011000761	Hide	2009	M	KF	29	15 10 54.9	26 29 00.4	Central	Z3	14	0.9945	0.0055
2011000762	Biopsy	2009	F	KF	38	14 28 03.9	26 38 08.0	GPS	H9	14	0.9946	0.0054
2011000763	Biopsy	2009	M	KF	38	14 27 14.7	26 38 30.9	GPS	Z3	14	0.9967	0.0033
2011000764	Biopsy	2009	M	KF	38	14 28 41.6	26 32 55.9	GPS	H9	14	0.9774	0.0226
2011000765	Biopsy	2009	M	KF	38	14 28 41.6	26 32 55.9	GPS	Z3	14	0.9959	0.0041
2011000766	Biopsy	2009	M	KF	38	14 28 41.6	26 32 55.9	GPS	Z1	0	na	na
2011000767	Biopsy	2009	F	KF	E	14 35 38.7	26 25 25.8	GPS	H9	14	0.9907	0.0093
2011000768	Biopsy	2009	M	KF	E	14 35 38.7	26 25 25.8	GPS	H9	13	0.9959	0.0041
2011000769	Biopsy	2009	F	KF	E	14 35 38.7	26 25 25.8	GPS	H9	14	0.997	0.003
2011000770	Biopsy	2009	M	LV	B	14 12 27.7	26 26 26.9	GPS	Z1	14	0.0059	0.9941
2011000772	Biopsy	2010	F	KF	F	15 58 06.9	25 50 33.2	GPS	Z3	14	0.9976	0.0024
2011000773	Biopsy	2010	F	KF	30	15 19 06.1	25 58 54.3	GPS		13	0.9934	0.0066
2011000775	Biopsy	2010	M	SI	G	16 53 54.7	23 35 44.0	GPS	H9	14	0.9063	0.0937
2011000776	Biopsy	2010	F	KF	F	15 54 08.7	25 57 37.4	GPS	Z3	14	0.996	0.004
2011000777	Biopsy	2010	F	KF	28	15 07 54.1	25 58 29.0	Central	H9	14	0.9816	0.0184
2011000778	Biopsy	2010	M	KF	28	15 07 54.1	25 58 29.0	Central	H9	0	na	na
2011000779	Biopsy	2010	M	KF	28	15 07 54.1	25 58 29.0	Central	H9	14	0.9963	0.0037
2011000780	Biopsy	2010	F	KF	30	15 22 37.3	26 02 37.6	GPS	H9	14	0.9933	0.0067
2011000781	Biopsy	2010	F	KF	30	15 22 37.3	26 02 37.6	GPS	H9	14	0.9864	0.0136
2011000782	Biopsy	2010	M	KF	F	15 58 17.4	25 48 36.7	GPS	Z3	0	na	na
2011000783	Biopsy	2010	M	KF	F	15 58 17.4	25 48 36.7	GPS	Z3	14	0.9971	0.0029
2011000784	Biopsy	2010	F	KF	F	15 58 17.4	25 48 36.7	GPS	Z3	14	0.9967	0.0033
2011000785	Biopsy	2010	M	KF	38	14 28 38.5	26 37 04.5	GPS	H9	14	0.9911	0.0089
2011000786	Biopsy	2010	F	KF	38	14 28 38.5	26 37 04.5	GPS	H9	14	0.9941	0.0059
2011000787	Biopsy	2010	M	KF	F	16 24 .15.0	25 48 35.0	GPS	Z3	14	0.9951	0.0049
2011000788	Biopsy	2010	F	KF	F	16 12 19.6	25 58 17.8	GPS	Z5	13	0.9673	0.0327
2011000789	Biopsy	2010	F	KF	F	16 12 21.6	25 58 17.9	GPS	Z5	14	0.9955	0.0045
2011000790	Biopsy	2010	M	KF	23	14 04 11.1	25 40 12.2	GPS	Z3	14	0.9961	0.0039
2011000791	Biopsy	2010	M	KF	F	15 58 44.5	25 48 30.2	GPS	Z3	14	0.9941	0.0059
2011000793	Biopsy	2011	M	KF	F	16 12 15.5	25 57 55.6	GPS	H1	14	0.9933	0.0067
2011000794	Biopsy	2011	M	KF	F	16 12 15.5	25 57 55.6	GPS	H9	14	0.9971	0.0029
2011000795	Biopsy	2011	M	KF	F	15 01 31.3	25 57 42.5	GPS	H9	14	0.9757	0.0243
2011000796	Biopsy	2011	F	KF	31	15 54 07.7	25 56 39.4	GPS	H9	14	0.9965	0.0035
2011000797	Biopsy	2011	M	KF	31	15 54 07.7	25 56 39.4	GPS	Z1	14	0.9969	0.0031
2011000798	Biopsy	2011	M	KF	31	15 54 07.7	25 56 39.4	GPS	H9	0	na	na
2011000799	Biopsy	2011	F	KF	31	16 01 19.2	25 56 19.4	GPS	H9	14	0.9974	0.0026
2011000800	Biopsy	2011	F	KF	31	16 01 19.1	25 56 19.2	GPS	H9	14	0.9965	0.0035
2011000801	Biopsy	2011	M	KF	30	15 20 57.4	25 59 16.1	GPS	H9	0	na	na
2011000802	Biopsy	2011	F	KF	F	15 53 30.3	25 50 13.7	GPS	Z3	14	0.9839	0.0161
2011000803	Biopsy	2011	F	KF	38	14 29 08.7	26 37 18.9	GPS	H9	14	0.9945	0.0055
2011000804	Biopsy	2011	F	KF	38	14 29 08.7	26 37 18.9	GPS	H9	14	0.9974	0.0026
2011000805	Hide	2011	M	KF	23	14 06 83.3	25 35 74.8	GPS	H9	14	0.9969	0.0031
2011000806	Hide	2012	M	KF	23	14 14 27.9	25 33 20.5	GPS	H9	13	0.9937	0.0063
2011000807	Hide	2012	M	KF	24	14 04 31.5	26 15 12.6	Central	H9	14	0.996	0.004
2011000809	Hide	2011	M	KF	25	13 55 48.6	26 35 46.2	Central	Z3	14	0.9934	0.0066
2011000810	Hide	2011	M	KF	25	13 55 48.6	26 35 46.2	Central	H9	14	0.9951	0.0049
2011000811	Hide	2012	M	KF	38	14 33 27.2	26 37 45.2	Central	H9	14	0.9935	0.0065
2011000812	Hide	2012	M	KF	39	14 27 25.0	26 55 40.4	Central	H9	14	0.9914	0.0086

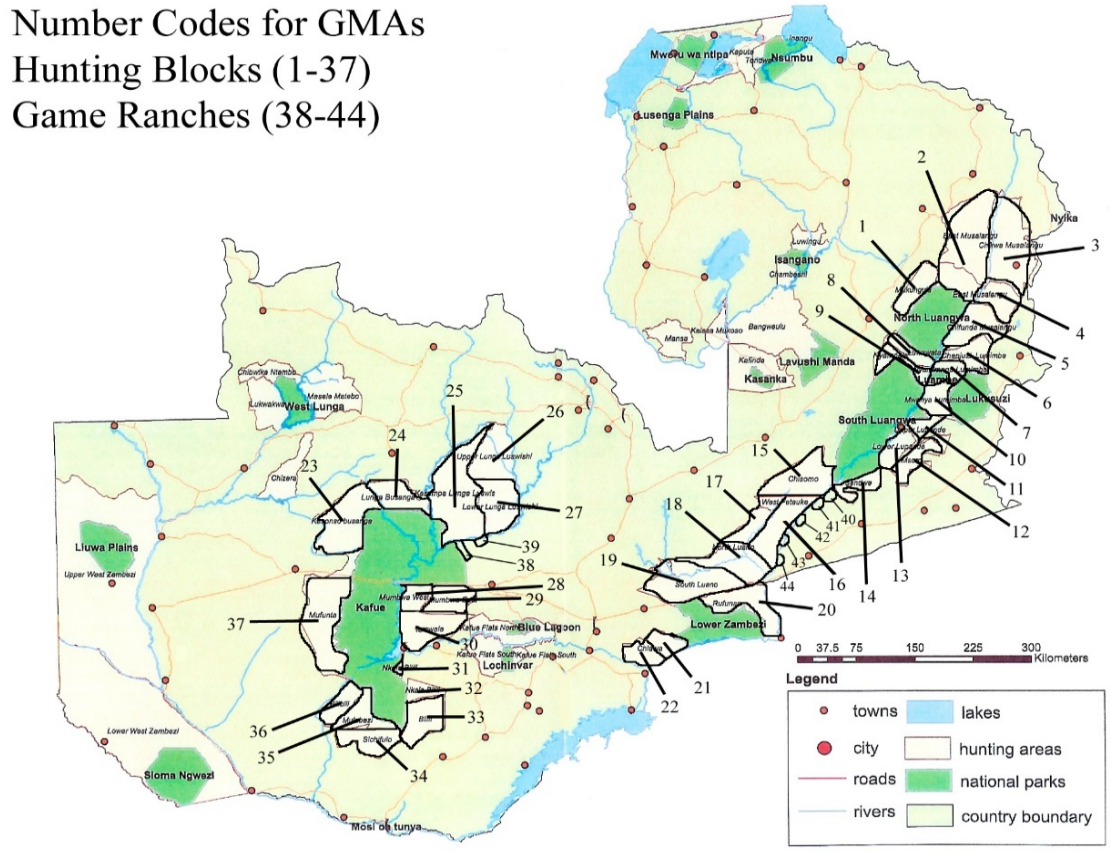
B.1.a: ZAMBIAN LIONS (PAGE 7 OF 8)

Sample Information			Animal Information						mtDNA	STRs	*STRUCTURE*	
TAMUID	Type	Date	Sex	Area	Location	S	E	Source	Haplotype	# Loci	Western	Eastern
2011000813	Hide	2012	M	KF	28	15 09 24.3	26 09 48.8	Central	H9	14	0.9901	0.0099
2011000814	Hide	2012	M	KF	28	15 09 24.3	26 09 48.8	Central	H9	14	0.9932	0.0068
2011000815	Hide	2012	M	KF	28	15 09 24.3	26 09 48.8	Central	H9	14	0.9939	0.0061
2011000816	Hide	2012	M	KF	28	15 09 24.3	26 09 48.8	Central	H9	14	0.9919	0.0081
2011000817	Hide	2012	M	KF	28	15 09 24.3	26 09 48.8	Central	Z3	14	0.994	0.006
2011000818	Hide	2012	M	KF	31	15 58 55.0	25 59 35.7	Central	Z3	14	0.991	0.009
2011000819	Hide	2012	M	KF	35	16 23 58.5	25 26 49.7	Central	H9	14	0.9701	0.0299
2011000820	Hide	2012	M	KF	35	16 23 58.5	25 26 49.7	Central	H9	14	0.9922	0.0078
2011000821	Hide	2012	M	LV	10	12 42 19.3	32 15 22.2	Central	Z1	13	0.0031	0.9969
2011000822	Hide	2012	M	LV	10	12 42 19.3	32 15 22.2	Central	Z1	14	0.0081	0.9919
2011000823	Hide	2012	M	LV	10	12 42 19.3	32 15 22.2	Central	Z1	14	0.0015	0.9985
2011000824	Hide	2012	M	LV	6	12 17 04.5	32 28 46.5	Central	Z1	14	0.0015	0.9985
2011000825	Hide	2012	M	LV	6	12 17 04.5	32 28 46.5	Central	Z1	14	0.0017	0.9983
2011000826	Hide	2012	M	LV	6	12 17 04.5	32 28 46.5	Central	Z1	14	0.0031	0.9969
2011000827	Hide	2012	M	LV	7	12 26 07.2	32 21 51.4	Central	Z1	14	0.0035	0.9965
2011000828	Hide	2012	M	LV	7	12 26 07.2	32 21 51.4	Central	H11	14	0.0015	0.9985
2011000829	Hide	2012	M	LV	7	12 26 07.2	32 21 51.4	Central	Z1	14	0.0065	0.9935
2011000830	Hide	2012	M	CO	40	14 05 46.7	30 43 42.5	Central	H11	14	0.1029	0.8971
2011000831	Hide	2012	F	CO	41	14 37 17.7	30 25 61.3	Central	H11	14	0.0442	0.9558
2011000832	Hide	2012	F	CO	41	14 37 17.7	30 25 61.3	Central	H11	14	0.0363	0.9637
2011000833	Hide	2012	M	KF	34	16 52 01.7	25 40 06.0	Central	Z3	14	0.9951	0.0049
2011000834	Hide	2012	M	CO	17	14 18 43.9	30 21 56.9	Central	Z1	14	0.0238	0.9762
2011000835	Hide	2012	M	LV	13	13 17 52.2	31 44 18.1	Central	Z1	14	0.0039	0.9961
2011000836	Hide	2012	M	LV	3	11 02 44.2	32 52 26.3	Central	H11	14	0.0025	0.9975
2011000837	Hide	2012	M	LV	3	11 02 44.2	32 52 26.3	Central	H11	14	0.0027	0.9973
2011000838	Hide	2012	M	LV	4	11 35 19.1	32 54 36.1	Central	Z1	14	0.0015	0.9985
2011000839	Hide	2010	M	LV	5	11 50 50.5	32 20 20.3	Central	H11	14	0.0025	0.9975
2011000840	Hide	2012	M	LV	5	11 50 50.5	32 20 20.3	Central	H11	14	0.0026	0.9974
2011000841	Hide	2012	M	LV	5	11 50 50.5	32 20 20.3	Central	H11	14	0.0037	0.9963
2011000842	Hide	2012	F	OUT	OUT	12 27 15.7	31 17 29.6	GPS	Z1	14	0.1647	0.8353
2011000844	Biopsy	2012	F	KF	F	15 52 14.2	25 44 49.4	GPS	Z3	14	0.9965	0.0035
2011000845	Biopsy	2012	F	KF	F	15 52 14.0	25 44 49.1	GPS	Z3	14	0.9951	0.0049
2011000846	Biopsy	2012	F	KF	F	15 52 14.0	25 44 49.1	GPS	Z3	14	0.9971	0.0029
2011000847	Biopsy	2012	M	KF	F	15 38 40.2	29 30 53.8	GPS	H11	14	0.7441	0.2559
2011000848	Biopsy	2012	F	ZA	D	15 38 33.8	29 31 12.2	GPS	H11	14	0.1135	0.8865
2011000849	Biopsy	2012	F	ZA	D	15 38 33.6	29 31 12.2	GPS	H11	14	0.0612	0.9388
2011000850	Biopsy	2012	F	LV	8	12 17 32.0	32 04 53.3	Central	H11	14	0.0045	0.9955
2011000851	Biopsy	2012		LV	9	12 37 57.3	32 01 39.7	GPS	Z1	14	0.0023	0.9977
2011000852	Biopsy	2012	F	LV	9	12 37 57.3	32 01 39.8	GPS	Z1	14	0.0018	0.9982
2011000854	Biopsy	2012	F	KF	F	16 09 09.9	26 03 28.5	GPS	Z5	14	0.9913	0.0087
2011000855	Biopsy	2012	F	KF	F	16 09 09.9	26 03 28.5	GPS	Z5	14	0.9933	0.0067
2011000856	Hide	2004	M	LV	5	11 54 44.1	32 26 02.9	GPS	Z1	14	0.0022	0.9978
2011000857	Hide	2004	M	LV	5	12 06 65.4	32 17 50.4	GPS	H11	14	0.0015	0.9985
2011000858	Hide	2004	M	LV	5	11 57 02.1	32 24 79.9	GPS	H11	14	0.0024	0.9976
2011000859	Hide	2004	M	LV	5	11 48 15.6	32 30 92.9	GPS	H11	14	0.0027	0.9973
2011000860	Hide	2004	M	LV	5	12 11 98.2	32 18 27.6	GPS	Z1	14	0.0021	0.9979
2011000861	Hide	2005	M	LV	6	12 17 04.5	32 28 46.5	Central	Z1	14	0.0034	0.9966
2011000862	Hide	2005	M	LV	7	12 26 07.2	32 21 51.4	Central	Z1	14	0.0023	0.9977
2011000863	Hide	2005	M	LV	7	12 26 07.2	32 21 51.4	Central	Z1	14	0.0037	0.9963
2011000864	Hide	2005	M	LV	7	12 26 07.2	32 21 51.4	Central	H11	14	0.0106	0.9894
2011000865	Hide	2004	M	LV	8	12 09 10.6	31 50 49.8	Central	Z1	14	0.0093	0.9907
2011000866	Hide	2004	M	LV	8	12 09 10.6	31 50 49.8	Central	Z1	14	0.0053	0.9947
2011000867	Hide	2004	M	LV	8	12 09 10.6	31 50 49.8	Central	H11	14	0.0035	0.9965
2011000868	Hide	2004	M	CO	42	14 12 31.7	30 36 34.6	Central	Z1	14	0.0213	0.9787
2011000869	Hide	2004	M	KF	23	14 16 13.7	25 26 08.2	Central	H9	14	0.9974	0.0026
2011000870	Hide	2004	M	KF	23	14 16 13.7	25 26 08.2	Central	H9	14	0.9974	0.0026

B.1.a: ZAMBIAN LIONS (PAGE 8 OF 8)

Sample Information			Animal Information						mtDNA	STRs	*STRUCTURE*	
TAMUID	Type	Date	Sex	Area	Location	S	E	Source	Haplotype	# Loci	Western	Eastern
2011000871	Hide	2003	M	LV	8	12 09 10.6	31 50 49.8	Central	Z1	14	0.0017	0.9983
2011000872	Hide	2003	M	LV	8	12 09 10.6	31 50 49.8	Central	Z1	14	0.0025	0.9975
2011000873	Hide	2003	M	LV	8	12 09 10.6	31 50 49.8	Central	H11	14	0.0027	0.9973
2011000874	Hide	2003	M	LV	8	12 09 10.6	31 50 49.8	Central	Z1	14	0.0097	0.9903
2011000875	Hide	2005	M	LV	8	12 09 10.6	31 50 49.8	Central	Z1	14	0.0017	0.9983
2011000876	Hide	2005	M	LV	8	12 09 10.6	31 50 49.8	Central	Z1	14	0.0015	0.9985
2011000877	Hide	2005	M	LV	8	12 09 10.6	31 50 49.8	Central	Z1	14	0.0011	0.9989
2011000878	Hide	2005	M	LV	2	11 02 44.2	32 52 26.3	Central	H11	14	0.5681	0.4319
2011000879	Hide	2005	M	LV	3	11 02 44.2	32 52 26.3	Central	Z1	14	0.0199	0.9801
2011000880	Hide	2005	M	LV	2	11 02 44.2	32 52 26.3	Central	Z1	14	0.0041	0.9959
2011000881	Hide	2005	M	LV	2	11 02 44.2	32 52 26.3	Central	Z1	14	0.0025	0.9975
2011000882	Hide	2005	M	KF	23	14 16 13.7	25 26 08.2	Central	H9	14	0.9959	0.0041
2011000883	Hide	2005	M	KF	23	14 16 13.7	25 26 08.2	Central	H9	14	0.9974	0.0026
2011000884	Hide	2005	M	CO	43	14 23 23.2	30 34 18.2	GPS	Z1	14	0.0455	0.9545
2011000885	Hide	2005	M	LV	8	12 09 10.6	31 50 49.8	Central	Z1	14	0.0286	0.9714
2011000886	Hide	2005	M	LV	5	11 50 50.5	32 20 20.3	Central	Z1	14	0.0029	0.9971
2011000887	Hide	2005	M	LV	5	11 50 50.5	32 20 20.3	Central	H11	14	0.0017	0.9983

Map numbers correspond with "Location" above



B.1.b: MODERN LIONS – NUCLEAR ANALYSIS (2 PAGES)

	Alternate IDs			Sampling	Hierarchical STRUCTURE Results					
Project ID	MD-1	MD-2	MD-3	Region	Country	Location	Continental	Subcontinental	Regional	Local
M_GIR_001	Ple50	India7		Northern	GIR	Gir Forest NP	Asia	Northern	India	GIR
M_GIR_002	Ple53	India5		Northern	GIR	Gir Forest NP	Asia	Northern	India	GIR
M_GIR_003	Ple58	India2		Northern	GIR	Gir Forest NP	Asia	Northern	India	GIR
M_GIR_004	Ple60	India10		Northern	GIR	Gir Forest NP	Asia	Northern	India	GIR
M_GIR_005	Ple67	India3		Northern	GIR	Gir Forest NP	Asia	Northern	India	GIR
M_GIR_006	Ple68	India1		Northern	GIR	Gir Forest NP	Asia	Northern	India	GIR
M_GIR_007	Ple69	India8		Northern	GIR	Gir Forest NP	Asia	Northern	India	GIR
M_GIR_008	Ple70	India9		Northern	GIR	Gir Forest NP	Asia	Northern	India	GIR
M_GIR_009	Ple71	India6		Northern	GIR	Gir Forest NP	Asia	Northern	India	GIR
M_GIR_010	Ple72	India4		Northern	GIR	Gir Forest NP	Asia	Northern	India	GIR
M_BEN_011		Benin01		Western	BEN	Pendjari NP	Africa	Western	West	WES
M_BEN_012		Benin02		Western	BEN	Pendjari NP	Africa	Western	West	WES
M_BEN_013		Benin03		Western	BEN	Pendjari NP	Africa	Western	West	WES
M_BEN_014		Benin04		Western	BEN	Pendjari NP	Africa	Western	West	WES
M_BEN_015		Benin05		Western	BEN	Pendjari NP	Africa	Western	West	WES
M_CAM_025		Cameroon10		Western	CAM	Bénoué Ecosystem	Africa	Western	West	CEN
M_CAM_026		Cameroon11		Western	CAM	Bénoué Ecosystem	Africa	Western	West	CEN
M_CAM_027		Cameroon12		Western	CAM	Bénoué Ecosystem	Africa	Western	West	CEN
M_CAM_016		Cameroon01		Western	CAM	Waza NP	Africa	Western	West	MID
M_CAM_017		Cameroon02		Western	CAM	Waza NP	Africa	Western	West	MID
M_CAM_018		Cameroon03		Western	CAM	Waza NP	Africa	Western	West	MID
M_CAM_019		Cameroon04		Western	CAM	Waza NP	Africa	Western	West	MID
M_CAM_020		Cameroon05		Western	CAM	Waza NP	Africa	Western	West	MID
M_CAM_021		Cameroon06		Western	CAM	Waza NP	Africa	Western	West	MID
M_CAM_022		Cameroon07		Western	CAM	Waza NP	Africa	Western	West	MID
M_CAM_023		Cameroon08		Western	CAM	Waza NP	Africa	Western	West	MID
M_CAM_024		Cameroon09		Western	CAM	Waza NP	Africa	Western	West	MID
M_CHD_028		Chad01		Western	CHD	Zakouma NP	Africa	Western	West	CEN
M_CHD_029		Chad02		Western	CHD	Zakouma NP	Africa	Western	West	CEN
M_CHD_030		Chad03		Western	CHD	Zakouma NP	Africa	Western	West	CEN
M_CHD_031		Chad04		Western	CHD	Zakouma NP	Africa	Western	West	CEN
M_DRC_032		DRC01		Western	DRC	Garamba NP	Africa	Western	West	CEN
M_DRC_033		DRC02		Western	DRC	Garamba NP	Africa	Western	West	CEN
M_DRC_034		DRC04		Western	DRC	Garamba NP	Africa	Western	West	CEN
M_DRC_035		DRC06		Western	DRC	Garamba NP	Africa	Western	West	CEN
M_DRC_132		DRC03		Western	DRC	Garamba NP	Africa	Southern	Southeast	UA
M_DRC_133		DRC05		Western	DRC	Garamba NP	Africa	UA	UA	UA
M_DRC_134		DRC07		Western	DRC	Garamba NP	Africa	UA	UA	UA
M_KEN_037		Kenya01		Eastern	KEN	Amboseli NP	Africa	Eastern	East	KEN
M_KEN_038		Kenya02		Eastern	KEN	Amboseli NP	Africa	Eastern	East	KEN
M_KEN_039		Kenya03		Eastern	KEN	Amboseli NP	Africa	Eastern	East	KEN
M_KEN_040		Kenya04		Eastern	KEN	Amboseli NP	Africa	Eastern	East	KEN
M_KEN_041		Kenya05		Eastern	KEN	Amboseli NP	Africa	Eastern	East	KEN
M_KEN_042		Kenya06		Eastern	KEN	Amboseli NP	Africa	Eastern	East	KEN
M_KEN_043		Kenya07		Eastern	KEN	Amboseli NP	Africa	Eastern	East	KEN
M_TAN_044	Ple235	Tanzania8		Eastern	TAN	Ngorongoro CA	Africa	Eastern	East	TAN
M_TAN_045	Ple239	Tanzania3		Eastern	TAN	Ngorongoro CA	Africa	Eastern	East	TAN
M_TAN_056	Ple349	Tanzania2		Eastern	TAN	Ngorongoro CA	Africa	Eastern	East	TAN
M_TAN_057	Ple359	Tanzania1		Eastern	TAN	Ngorongoro CA	Africa	Eastern	East	TAN
M_TAN_058	Ple490	Tanzania6		Eastern	TAN	Ngorongoro CA	Africa	Eastern	East	TAN
M_TAN_059	Ple493	Tanzania10		Eastern	TAN	Ngorongoro CA	Africa	Eastern	East	TAN
M_TAN_060	Ple508	Tanzania5		Eastern	TAN	Ngorongoro CA	Africa	Eastern	East	TAN
M_TAN_061	Ple510	Tanzania7		Eastern	TAN	Ngorongoro CA	Africa	Eastern	East	TAN
M_TAN_062	Ple539	Tanzania4		Eastern	TAN	Ngorongoro CA	Africa	Eastern	East	TAN
M_TAN_063	Ple572	Tanzania9		Eastern	TAN	Ngorongoro CA	Africa	Eastern	East	TAN
M_TAN_046	Ple276	Tanzania19		Eastern	TAN	Serengeti NP	Africa	Eastern	East	TAN
M_TAN_047	Ple279	Tanzania12		Eastern	TAN	Serengeti NP	Africa	Eastern	East	TAN
M_TAN_048	Ple289	Tanzania20		Eastern	TAN	Serengeti NP	Africa	Eastern	East	TAN
M_TAN_049	Ple301	Tanzania11		Eastern	TAN	Serengeti NP	Africa	Eastern	East	TAN
M_TAN_050	Ple305	Tanzania13		Eastern	TAN	Serengeti NP	Africa	Eastern	East	TAN
M_TAN_051	Ple307	Tanzania14		Eastern	TAN	Serengeti NP	Africa	Eastern	East	TAN
M_TAN_052	Ple311	Tanzania15		Eastern	TAN	Serengeti NP	Africa	Eastern	East	TAN
M_TAN_053	Ple313	Tanzania16		Eastern	TAN	Serengeti NP	Africa	Eastern	East	TAN
M_TAN_054	Ple315	Tanzania17		Eastern	TAN	Serengeti NP	Africa	Eastern	East	TAN
M_TAN_055	Ple317	Tanzania18		Eastern	TAN	Serengeti NP	Africa	Eastern	East	TAN
M_TAN_036				Eastern	TAN	Tanzania	Africa	Western	West	CEN
M_TAN_135				Eastern	TAN	Tanzania	Africa	Southern	Southeast	UA
M_ZAM_078		Zambia9	2011000317	Southern	ZAM	Kafue NP	Africa	Southern	Southwest	ZAW

B.1.b: MODERN LIONS – NUCLEAR ANALYSIS (PAGE 2 OF 2)

	Alternate IDs			Sampling	Hierarchical STRUCTURE Results					
Project ID	MD-1	MD-2	MD-3	Region	Country	Location	Continental	Subcontinental	Regional	Local
M_ZAM_079			2011000325	Southern	ZAM	Kafue NP	Africa	Southern	Southwest	ZAW
M_ZAM_080			2011000397	Southern	ZAM	Kafue NP	Africa	Southern	Southwest	ZAW
M_ZAM_081			2011000481	Southern	ZAM	Kafue NP	Africa	Southern	Southwest	ZAW
M_ZAM_082			2011000482	Southern	ZAM	Kafue NP	Africa	Southern	Southwest	ZAW
M_ZAM_083			2011000738	Southern	ZAM	Kafue NP	Africa	Southern	Southwest	ZAW
M_ZAM_084			2011000740	Southern	ZAM	Kafue NP	Africa	Southern	Southwest	ZAW
M_ZAM_085			2011000746	Southern	ZAM	Kafue NP	Africa	Southern	Southwest	ZAW
M_ZAM_086			2011000748	Southern	ZAM	Kafue NP	Africa	Southern	Southwest	ZAW
M_ZAM_087			2011000763	Southern	ZAM	Kafue NP	Africa	Southern	Southwest	ZAW
M_ZAM_088			2011000784	Southern	ZAM	Kafue NP	Africa	Southern	Southwest	ZAW
M_ZAM_089			2011000817	Southern	ZAM	Kafue NP	Africa	Southern	Southwest	ZAW
M_ZAM_090			2011000819	Southern	ZAM	Kafue NP	Africa	Southern	Southwest	ZAW
M_ZAM_115			2011000310	Southern	ZAM	Kafue NP	Africa	Southern	Southwest	ETO
M_ZAM_128			2011000401	Southern	ZAM	Kafue NP	Africa	Southern	UA	UA
M_ZAM_129			2011000409	Southern	ZAM	Kafue NP	Africa	Southern	UA	UA
M_ZAM_131			2011000883	Southern	ZAM	Kafue NP	Africa	Southern	UA	UA
M_ZAM_064			2011000286	Southern	ZAM	Luangwa Valley	Africa	Southern	Southeast	ZAE
M_ZAM_065		Zambia5	2011000298	Southern	ZAM	Luangwa Valley	Africa	Southern	Southeast	ZAE
M_ZAM_066		Zambia6	2011000299	Southern	ZAM	Luangwa Valley	Africa	Southern	Southeast	ZAE
M_ZAM_067		Zambia2	2011000300	Southern	ZAM	Luangwa Valley	Africa	Southern	Southeast	ZAE
M_ZAM_068		Zambia3	2011000301	Southern	ZAM	Luangwa Valley	Africa	Southern	Southeast	ZAE
M_ZAM_069		Zambia1	2011000302	Southern	ZAM	Luangwa Valley	Africa	Southern	Southeast	ZAE
M_ZAM_070		Zambia4	2011000305	Southern	ZAM	Luangwa Valley	Africa	Southern	Southeast	ZAE
M_ZAM_071			2011000312	Southern	ZAM	Luangwa Valley	Africa	Southern	Southeast	ZAE
M_ZAM_072		Zambia7	2011000324	Southern	ZAM	Luangwa Valley	Africa	Southern	Southeast	ZAE
M_ZAM_073		Zambia8	2011000353	Southern	ZAM	Luangwa Valley	Africa	Southern	Southeast	ZAE
M_ZAM_074			2011000418	Southern	ZAM	Luangwa Valley	Africa	Southern	Southeast	ZAE
M_ZAM_075			2011000699	Southern	ZAM	Luangwa Valley	Africa	Southern	Southeast	ZAE
M_ZAM_076			2011000706	Southern	ZAM	Luangwa Valley	Africa	Southern	Southeast	ZAE
M_ZAM_077			2011000849	Southern	ZAM	Luangwa Valley	Africa	Southern	Southeast	ZAE
M_ZAM_094			2011000881	Southern	ZAM	Luangwa Valley	Africa	Southern	Southeast	KRU
M_ZAM_126			2011000288	Southern	ZAM	Luangwa Valley	Africa	Southern	Southeast	UA
M_ZAM_127			2011000399	Southern	ZAM	Luangwa Valley	Africa	Southern	Southeast	UA
M_NAM_116	Ple402	Namibia1		Southern	NAM	Etoshia NP	Africa	Southern	Southwest	ETO
M_NAM_117	Ple406	Namibia2		Southern	NAM	Etoshia NP	Africa	Southern	Southwest	ETO
M_NAM_118	Ple409	Namibia3		Southern	NAM	Etoshia NP	Africa	Southern	Southwest	ETO
M_NAM_119	Ple411	Namibia4		Southern	NAM	Etoshia NP	Africa	Southern	Southwest	ETO
M_NAM_120	Ple425	Namibia5		Southern	NAM	Etoshia NP	Africa	Southern	Southwest	ETO
M_NAM_121	Ple430	Namibia6		Southern	NAM	Etoshia NP	Africa	Southern	Southwest	ETO
M_NAM_122	Ple437	Namibia8		Southern	NAM	Etoshia NP	Africa	Southern	Southwest	ETO
M_NAM_123	Ple439	Namibia9		Southern	NAM	Etoshia NP	Africa	Southern	Southwest	ETO
M_NAM_124	Ple443	Namibia7		Southern	NAM	Etoshia NP	Africa	Southern	Southwest	ETO
M_NAM_125	Ple448	Namibia10		Southern	NAM	Etoshia NP	Africa	Southern	Southwest	ETO
M_RSA_105	Ple701	RSA1		Southern	RSA	Kalahari-Gemsbok NP	Africa	Southern	Southwest	KAL
M_RSA_106	Ple705	RSA2		Southern	RSA	Kalahari-Gemsbok NP	Africa	Southern	Southwest	KAL
M_RSA_107	Ple707	RSA3		Southern	RSA	Kalahari-Gemsbok NP	Africa	Southern	Southwest	KAL
M_RSA_108	Ple708	RSA4		Southern	RSA	Kalahari-Gemsbok NP	Africa	Southern	Southwest	KAL
M_RSA_109	Ple710	RSA5		Southern	RSA	Kalahari-Gemsbok NP	Africa	Southern	Southwest	KAL
M_RSA_110	Ple711	RSA6		Southern	RSA	Kalahari-Gemsbok NP	Africa	Southern	Southwest	KAL
M_RSA_111	Ple713	RSA7		Southern	RSA	Kalahari-Gemsbok NP	Africa	Southern	Southwest	KAL
M_RSA_112	Ple720	RSA8		Southern	RSA	Kalahari-Gemsbok NP	Africa	Southern	Southwest	KAL
M_RSA_113	Ple721	RSA9		Southern	RSA	Kalahari-Gemsbok NP	Africa	Southern	Southwest	KAL
M_RSA_114	Ple724	RSA10		Southern	RSA	Kalahari-Gemsbok NP	Africa	Southern	Southwest	KAL
M_RSA_095	Ple150	RSA11		Southern	RSA	Kruger NP	Africa	Southern	South	KRU
M_RSA_096	Ple154	RSA12		Southern	RSA	Kruger NP	Africa	Southern	South	KRU
M_RSA_097	Ple155	RSA13		Southern	RSA	Kruger NP	Africa	Southern	South	KRU
M_RSA_098	Ple157	RSA14		Southern	RSA	Kruger NP	Africa	Southern	South	KRU
M_RSA_099	Ple165	RSA15		Southern	RSA	Kruger NP	Africa	Southern	South	KRU
M_RSA_100	Ple168	RSA16		Southern	RSA	Kruger NP	Africa	Southern	South	KRU
M_RSA_101	Ple171	RSA17		Southern	RSA	Kruger NP	Africa	Southern	South	KRU
M_RSA_102	Ple174	RSA18		Southern	RSA	Kruger NP	Africa	Southern	South	KRU
M_RSA_103	Ple179	RSA19		Southern	RSA	Kruger NP	Africa	Southern	South	KRU
M_RSA_104	Ple181b	RSA20		Southern	RSA	Kruger NP	Africa	Southern	South	KRU
M_RSA_091				Southern	RSA	South Africa	Africa	Southern	Southeast	KRU
M_RSA_092				Southern	RSA	South Africa	Africa	Southern	Southeast	KRU
M_RSA_093				Southern	RSA	South Africa	Africa	Southern	Southeast	KRU
M_RSA_130				Southern	RSA	South Africa	Africa	Southern	UA	UA

B.1.c: MODERN LIONS – MITOCHONDRIAL ANALYSIS

				Sampling			Original	NGS Coverage			mtDNA Sequence Variation			
Project ID	Accession #	Haplotype	Clade	Region	Country	Location	Material	Protocol	DNA	mtDNA	% Called	%Missing	REF	ALT	?
M_ZAM_T256	This study	Hap_39	M	Southern	ZAM	Kafue	Hide	Tissue	1.7	72.0	99.9%	0.1%	1811	0	1
M_ZAM_T429	This study	Hap_39	M	Southern	ZAM	Kafue	Hide	Tissue	1.3	25.1	98.1%	1.9%	1777	0	35
M_TAN_135	This study	Hap_37	M	Eastern	TAN	Tanzania	Bone	Bone	1.6	331.0	99.9%	0.1%	1790	21	1
M_BEN_011	KP001497	Hap_8	W	Western	BEN	Pendjari NP									
M_CAM_020	KP001502	Hap_21	W	Western	CAM	Waza NP									
M_CAM_025	KP001493	Hap_22	W	Western	CAM	Bénoué Ecosystem									
M_DRC_033	KP001506	Hap_17	W	Western	DRC	Garamba NP									
M_DRC_KP494	KP001494	Hap_20	W	Western	DRC	Captive (i)									
M_ETH_KP495	KP001495	Hap_23	W	Eastern	ETH	Ethiopia									
M_GIR_009	KP001501	Hap_6	W	Northern	GIR	Gir Forest NP									
M_GIR_KC784	KC834784	Hap_5	W	Northern	GIR	India									
M_KEN_KP498	KP001498	Hap_66	M	Eastern	KEN	Tsavo East NP									
M_NAM_KP496	KP001496	Hap_74	S	Southern	NAM	Captive									
M_NAM_KP504	KP001504	Hap_86	S	Southern	NAM	Eastern Etosha									
M_PLE_KP262	KP202262	Hap_19	W	Western	UNK	Captive									
M_RSA_KP500	KP001500	Hap_71	S	Southern	RSA	Kruger NP: Timbavati									
M_SOM_KP499	KP001499	Hap_33	E	Eastern	SOM	Captive (vi)									
M_ZAM_KP503	KP001503	Hap_38	M	Southern	ZAM	Mpika town									
M_ZAM_KP505	KP001505	Hap_39	M	Southern	ZAM	Mulobezi town									

B.1.d: HISTORICAL LIONS – LOCATION INFORMATION (2 PAGES) – Dates in *italics* were extrapolated from expedition.

Project ID	Museum ID	Sampling Region	Current Country	Year	Latitude	Longitude	Location (from Museum Record)	Collector/Expedition
H_BOT_001	AMNH_119594	Southern	BOT	*1930*	-19.4040	23.5040	Ngamiland	Vernay-Lang Kalahari Expedition
H_BOT_002	AMNH_119595	Southern	BOT	*1930*	-19.4040	23.5040	Ngamiland	Vernay-Lang Kalahari Expedition
H_COG_003	AMNH_119870	Western	COG	*1949*	-4.2548	15.2518	French Equetorial Africa, Environsde Brazzaville	MacLatchy-Malbrant
H_GAB_004	AMNH_119871	Western	GAB	*1949*	-0.0925	11.9486	French Equetorial Africa, Booue Gabon	MacLatchy-Malbrant
H_CPT_005	AMNH_13904	Captive	CAP	1898			Captive	
H_CPT_006	AMNH_13998	Captive	CAP	1898			Captive	
H_CPT_007	AMNH_14027	Captive	CAP	1895			Captive	
H_CPT_008	AMNH_14028	Captive	CAP	1895			Captive	
H_CPT_009	AMNH_14034	Captive	CAP	1896			Captive	
H_ZIM_010	AMNH_161011	Southern	ZAM	1946	-14.0000	27.0000	Rhodesia: Namwaka	T.D. Carter
H_MWI_011	AMNH_161732	Southern	MWI	1946	-13.2734	33.6373	Nyasaland: Ntchisi to Chibotela	H.E. Anthony
H_COG_012	AMNH_17274	Western	COG	*<1920*	-4.2548	15.2518	Congo Region	Mr. Glove
H_COG_013	AMNH_17275	Western	COG	*<1920*	-4.2548	15.2518	Congo Region	Mr. Glove, Gift of the Century Co.
H_NAM_014	AMNH_19181	Southern	NAM	*<1913*	-19.0857	16.3993	German-South-West Africa	H.H. Vogelsang Collection (2798/1211)
H_CPT_015	AMNH_24249	Captive	CAP	1905			Captive	Original No. 1440
H_KEN_016	AMNH_27769	Eastern	KEN	1906	-0.1000	36.1000	British East Africa	Tjader Expedition
H_RSA_017	AMNH_28151	Southern	RSA	*1905*	-29.0000	24.0000	South Africa	Richard Douglas Collection
H_KEN_018	AMNH_30240	Eastern	KEN	*1912*	-0.1000	36.1000	British East Africa	Paul J. Rainey Expedition
H_KEN_019	AMNH_30241	Eastern	KEN	*1912*	-0.1000	36.1000	British East Africa	Paul J. Rainey Expedition
H_KEN_020	AMNH_30242	Eastern	KEN	*1912*	-0.1000	36.1000	British East Africa	Paul J. Rainey Expedition
H_KEN_021	AMNH_30243	Eastern	KEN	*1912*	-0.1000	36.1000	British East Africa	Paul J. Rainey Expedition
H_KEN_022	AMNH_30244	Eastern	KEN	*1912*	-0.1000	36.1000	British East Africa	Paul J. Rainey Expedition
H_KEN_023	AMNH_30245	Eastern	KEN	*1912*	-0.1000	36.1000	British East Africa	Paul J. Rainey Expedition
H_KEN_024	AMNH_30246	Eastern	KEN	*1912*	-0.1000	36.1000	British East Africa	Paul J. Rainey Expedition
H_KEN_025	AMNH_30247	Eastern	KEN	*1912*	-0.1000	36.1000	British East Africa	Paul J. Rainey Expedition
H_KEN_026	AMNH_30248	Eastern	KEN	*1912*	-0.1000	36.1000	British East Africa	Paul J. Rainey Expedition
H_CPT_027	AMNH_35472	Captive	CAP	1912			Captive	
H_KEN_028	AMNH_36420	Eastern	KEN	*1912*	-0.1000	36.1000	British East Africa	Paul J. Rainey Expedition
H_KEN_029	AMNH_36421	Eastern	KEN	*1912*	-0.1000	36.1000	British East Africa	Paul J. Rainey Expedition
H_CPT_030	AMNH_403	Captive	CAP	1886			Captive	
H_DRC_031	AMNH_52070	Western	DRC	1911	3.7327	29.7164	Belgian Congo: Faradje	American Museum Congo Expedition
H_DRC_032	AMNH_52071	Western	DRC	1911	3.7327	29.7164	Belgian Congo: Faradje	American Museum Congo Expedition
H_DRC_033	AMNH_52072	Western	DRC	1911	3.7327	29.7164	Belgian Congo: Faradje	American Museum Congo Expedition
H_DRC_034	AMNH_52073	Western	DRC	1911	3.7327	29.7164	Belgian Congo: Faradje	American Museum Congo Expedition
H_DRC_035	AMNH_52074	Western	DRC	1911	3.7327	29.7164	Belgian Congo: Faradje	American Museum Congo Expedition
H_DRC_036	AMNH_52075	Western	DRC	1911	3.7327	29.7164	Belgian Congo: Faradje	American Museum Congo Expedition
H_DRC_037	AMNH_52076	Western	DRC	1911	3.7327	29.7164	Belgian Congo: Faradje	American Museum Congo Expedition
H_DRC_038	AMNH_52077	Western	DRC	1912	3.7327	29.7164	Belgian Congo: Faradje	American Museum Congo Expedition
H_DRC_039	AMNH_52078	Western	DRC	1912	3.7327	29.7164	Belgian Congo: Faradje	American Museum Congo Expedition
H_DRC_040	AMNH_52079	Western	DRC	1912	3.7327	29.7164	Belgian Congo: Faradje	American Museum Congo Expedition
H_DRC_041	AMNH_52080	Western	DRC	1912	3.7327	29.7164	Belgian Congo: Faradje	American Museum Congo Expedition
H_DRC_042	AMNH_52081	Western	DRC	1912	3.7327	29.7164	Belgian Congo: Faradje	American Museum Congo Expedition
H_DRC_043	AMNH_52082	Western	DRC	1912	3.7327	29.7164	Belgian Congo: Faradje	American Museum Congo Expedition
H_KEN_044	AMNH_54370	Eastern	KEN	1912	0.5160	35.3234	Kenya	Akeley Expedition to British East Africa
H_KEN_045	AMNH_54371	Eastern	KEN	1912	0.5160	35.3234	Kenya	Akeley Expedition to British East Africa
H_KEN_046	AMNH_54372	Eastern	KEN	1912	0.5160	35.3234	Kenya	Akeley Expedition to British East Africa
H_KEN_047	AMNH_54393	Eastern	KEN	1912	0.5160	35.3234	Kenya: Gyasiu Nghishu Plateau	Akeley Expedition to British East Africa
H_KEN_048	AMNH_54394	Eastern	KEN	1912	0.5160	35.3234	Kenya	Akeley Expedition to British East Africa
H_KEN_049	AMNH_54395	Eastern	KEN	1912	0.5160	35.3234	Kenya: Gyasiu Nghishu Plateau	Akeley Expedition to British East Africa
H_GIR_050	AMNH_54995	Northern	GIR	1929	21.1240	70.8217	India: Junagadh State, Gir Forest	Faunthorpe-Vernay Expedition
H_GIR_051	AMNH_54996	Northern	GIR	1929	21.1240	70.8217	India: Junagadh State, Gir Forest	Faunthorpe-Vernay Expedition
H_CPT_052	AMNH_6260	Captive	CAP	1893			Captive	
H_CPT_053	AMNH_6282	Captive	CAP	1889			Captive	
H_GIR_054	AMNH_63955	Northern	GIR	1906	21.1240	70.8217	India	
H_CPT_055	AMNH_65	Captive	CAP	*1860*			Captive	Maximillian Collection
H_CPT_056	AMNH_70171	Captive	CAP	1924			Captive	
H_KEN_057	AMNH_70347	Eastern	KEN	1922	-0.1000	36.1000	British East Africa	Wild caught: Donated to NY Zoo by Buffalo Jones
H_ANG_058	AMNH_80609	Southern	ANG	1925	-14.4699	16.2911	Angola: Capelongo	Vernay Angola Expedition/Lang Collection
H_RSA_059	AMNH_81836	Southern	RSA	*1930*	-25.0000	29.0000	Transvaal	Lang-deLaporte/Lang Collection
H_RSA_060	AMNH_81837	Southern	RSA	*1930*	-25.0000	29.0000	Transvaal	Lang-deLaporte/Lang Collection
H_RSA_061	AMNH_81839	Southern	RSA	*1930*	-25.0000	29.0000	Transvaal	Lang-deLaporte/Lang Collection
H_RSA_062	AMNH_81840	Southern	RSA	*1930*	-25.0000	29.0000	Transvaal	Lang-deLaporte/Lang Collection
H_RSA_063	AMNH_81841	Southern	RSA	*1930*	-25.0000	29.0000	Transvaal	Lang-deLaporte/Lang Collection
H_RSA_064	AMNH_81842	Southern	RSA	*1930*	-25.0000	29.0000	Transvaal	Lang-deLaporte/Lang Collection
H_RSA_065	AMNH_81843	Southern	RSA	*1930*	-25.0000	29.0000	Transvaal	Lang-deLaporte/Lang Collection
H_RSA_066	AMNH_81844	Southern	RSA	*1930*	-25.0000	29.0000	Transvaal	Lang-deLaporte/Lang Collection
H_CAR_067	AMNH_83410	Western	CAR	1924	6.9846	18.9116	French Equatorial Africa, NE Section, Ubongui-Churi	Mrs. Martha Bliven
H_CPT_068	AMNH_8355	Captive	CAP	1895			Captive	
H_BOT_069	AMNH_83617	Southern	BOT	*1930*	-19.1492	23.7915	Kalahari	Vernay-Lang Kalahari Expedition
H_BOT_070	AMNH_83618	Southern	BOT	*1930*	-19.1492	23.7915	Bechuanaland Protectorate: Kwaai, R. Machaba	Vernay-Lang Kalahari Expedition
H_BOT_071	AMNH_83619	Southern	BOT	*1930*	-19.1492	23.7915	Bechuanaland Protectorate: Kwaai, R. Machaba	Vernay-Lang Kalahari Expedition
H_BOT_072	AMNH_83620	Southern	BOT	*1930*	-19.1492	23.7915	Bechuanaland Protectorate: Kwaai, R. Machaba	Vernay-Lang Kalahari Expedition
H_BOT_073	AMNH_83621	Southern	BOT	*1930*	-19.1492	23.7915	Bechuanaland Protectorate: Kwaai, R. Machaba	Vernay-Lang Kalahari Expedition
H_BOT_074	AMNH_83622	Southern	BOT	*1930*	-19.1492	23.7915	Bechuanaland Protectorate: Kwaai, R. Machaba	Vernay-Lang Kalahari Expedition
H_BOT_075	AMNH_83623	Southern	BOT	*1930*	-19.1492	23.7915	Bechuanaland Protectorate: Kwaai, R. Machaba	Vernay-Lang Kalahari Expedition
H_BOT_076	AMNH_83624	Southern	BOT	*1930*	-19.7000	26.4000	Bechuanaland Protectorate: Nkate	Vernay-Lang Kalahari Expedition
H_BOT_077	AMNH_83625	Southern	BOT	*1930*	-18.7167	24.3500	Bechuanaland Protectorate: Tsotsoroga Pan	Vernay-Lang Kalahari Expedition
H_CPT_078	AMNH_8364	Captive	CAP	1895			Captive	
H_TAN_079	AMNH_85140	Eastern	TAN	1928	-6.2582	37.7166	Tanganyika Territory: Serengeti Plains	Carlisle-Clark African Expedition
H_TAN_080	AMNH_85141-L	Eastern	TAN	1928	-6.2582	37.7166	Tanganyika Territory: Serengeti Plains	Carlisle-Clark African Expedition
H_TAN_081	AMNH_85141-N	Eastern	TAN	1928	-6.2582	37.7166	Tanganyika Territory: Serengeti Plains	Carlisle-Clark African Expedition
H_TAN_082	AMNH_85142-L	Eastern	TAN	1928	-6.2582	37.7166	Tanganyika Territory: Serengeti Plains	Carlisle-Clark African Expedition
H_TAN_083	AMNH_85142-N	Eastern	TAN	1928	-6.2582	37.7166	Tanganyika Territory: Serengeti Plains	Carlisle-Clark African Expedition
H_TAN_084	AMNH_85143	Eastern	TAN	1928	-6.2582	37.7166	Tanganyika Territory: Serengeti Plains	Carlisle-Clark African Expedition
H_TAN_085	AMNH_85144	Eastern	TAN	1928	-6.2582	37.7166	Tanganyika Territory: Serengeti Plains	Carlisle-Clark African Expedition
H_TAN_086	AMNH_85145	Eastern	TAN	1928	-6.2582	37.7166	Tanganyika Territory: Serengeti Plains	Carlisle-Clark African Expedition
H_TAN_087	AMNH_85146	Eastern	TAN	1928	-6.2582	37.7166	Tanganyika Territory: Serengeti Plains	Carlisle-Clark African Expedition

B.1.d: HISTORICAL LIONS – LOCATION INFORMATION (PAGE 2 OF 2) – Dates in *italics* were extrapolated from expedition.

Project ID	Museum ID	Sampling Region	Current Country	Year	Latitude	Longitude	Location (from Museum Record)	Collector/Expedition
H_TAN_088	AMNH_85147	Eastern	TAN	1928	-6.2582	37.7166	Tanganyika Territory: Serengeti Plains	Carlisle-Clark African Expedition
H_TAN_089	AMNH_85148	Eastern	TAN	1928	-6.2582	37.7166	Tanganyika Territory: Serengeti Plains	Carlisle-Clark African Expedition
H_TAN_090	AMNH_85149	Eastern	TAN	1928	-6.2582	37.7166	Tanganyika Territory: Serengeti Plains	Carlisle-Clark African Expedition
H_KEN_091	AMNH_88632	Eastern	KEN	*1912*	-0.1000	36.1000	British East Africa	3rd African Expedition of the American Museum
H_KEN_092	AMNH_88633	Eastern	KEN	*1912*	-0.1000	36.1000	British East Africa	3rd African Expedition of the American Museum
H_KEN_093	AMNH_88634	Eastern	KEN	*1912*	-0.1000	36.1000	British East Africa	3rd African Expedition of the American Museum
H_KEN_094	AMNH_88635	Eastern	KEN	*1912*	-0.1000	36.1000	British East Africa	3rd African Expedition of the American Museum
H_KEN_095	AMNH_88636	Eastern	KEN	*1912*	-0.1000	36.1000	British East Africa	3rd African Expedition of the American Museum
H_KEN_096	AMNH_88637	Eastern	KEN	*1912*	-0.1000	36.1000	British East Africa	3rd African Expedition of the American Museum
H_CPT_097	CM_1461	Captive	CAP				Captive	Pittsburgh Zoological Gargen
H_CPT_098	CM_1564	Captive	CAP	1908			Captive	Pittsburgh Zoological Gargen
H_CPT_099	CM_1565	Captive	CAP	1908			Captive	Pittsburgh Zoological Gargen
H_CPT_100	CM_1825	Captive	CAP				Captive	Pittsburgh Zoological Gargen
H_UNK_101	CM_184	Unknown	UNK					The Klondike Museum
H_UNK_102	CM_185	Unknown	UNK					The Klondike Museum
H_UNK_103	CM_31	Unknown	UNK					Webster, F S
H_ETH_104	CM_5868	Eastern	ETH	1912	7.5000	40.0000	Abyssinia, 9-20 MI UP THIKA RIVER*	Frick, C
H_TAN_105	CM_5897	Eastern	TAN	1927	-6.0000	35.0000	Tanzania	Arbuthnot, T H
H_TAN_106	CM_5898	Eastern	TAN	1927	-6.0000	35.0000	Tanzania	Arbuthnot, T H
H_TAN_107	CM_5899	Eastern	TAN	1927	-6.0000	35.0000	Tanzania	Arbuthnot, T H
H_TAN_108	FMNH_127836	Eastern	TAN	1928	-2.4364	34.8210	Mara Region, Bariadi District, Serengeti Plains, Seronera	Cudahy-Massee Expedition
H_TAN_109	FMNH_127837	Eastern	TAN	1928	-2.4364	34.8210	Mara Region, Bariadi District, Serengeti Plains, Seronera	Cudahy-Massee Expedition
H_TAN_110	FMNH_127838	Eastern	TAN	1928	-2.4364	34.8210	Mara Region, Bariadi District, Serengeti Plains, Seronera	Cudahy-Massee Expedition
H_TAN_111	FMNH_127839	Eastern	TAN	1928	-2.4364	34.8210	Mara Region, Bariadi District, Serengeti Plains, Seronera	Cudahy-Massee Expedition
H_TAN_112	FMNH_127840	Eastern	TAN		-6.0000	35.0000	Tanzania	
H_SOM_113	FMNH_1443	Eastern	SOM	1896	10.0000	44.0000	Woqooyi Galbeed, Bannaanko Tuuyo ("Tuyo Plain")	D. G. Elliot
H_KEN_114	FMNH_20756	Eastern	KEN	1905	-1.4613	36.9827	Eastern Prov, Machakos Dist, Athi Plains	Akeley Expedition to British East Africa
H_KEN_115	FMNH_20757	Eastern	KEN	1905	-1.4613	36.9827	Eastern Prov, Machakos Dist, Athi Plains	Akeley Expedition to British East Africa
H_KEN_116	FMNH_20758	Eastern	KEN	1905	-1.4613	36.9827	Eastern Prov, Machakos Dist, Athi Plains	Akeley Expedition to British East Africa
H_KEN_117	FMNH_20760	Eastern	KEN	1905	-1.4613	36.9827	Eastern Prov, Machakos Dist, Athi Plains	Akeley Expedition to British East Africa
H_KEN_118	FMNH_20762	Eastern	KEN	1905	-1.4613	36.9827	Eastern Prov, Machakos Dist, Athi Plains	Akeley Expedition to British East Africa
H_SDN_119	FMNH_30778	Western	SDN	1928	13.0000	35.5000	Kassala, Rahad R, Abid, Ethiopian border, 20 mi NW	J. E. Baum
H_GIR_120	FMNH_31121	Northern	GIR	1929	21.1240	70.8217	Kathiawar, Gir Forest	Faunthorpe-Vernay Expedition
H_TAN_121	FMNH_33479	Eastern	TAN	1929	-3.0000	35.0000	Serengeti Plains	C. J. Albrecht
H_TAN_122	FMNH_33480	Eastern	TAN	1929	-3.0000	35.0000	Serengeti Plains	C. J. Albrecht
H_TAN_123	FMNH_35131	Eastern	TAN	1930	-3.0000	35.0000	Serengeti Plains	M. Field
H_TAN_124	FMNH_35132	Eastern	TAN	1930	-3.0000	35.0000	Serengeti Plains	M. Field
H_TAN_125	FMNH_35133	Eastern	TAN	*1930*	-3.0000	35.0000	Serengeti Plains	M. Field
H_TAN_126	FMNH_35134	Eastern	TAN	*1930*	-3.0000	35.0000	Serengeti Plains	M. Field
H_BOT_127	FMNH_35739	Southern	BOT	1930	-22.5500	23.2500	Kaotwe Pan, ca 45 mi SE of Gomodimo Pan	Vernay-Lang Kalahari Expedition
H_BOT_128	FMNH_35740	Southern	BOT	1930	-19.1953	24.0070	Mababe Flats	Vernay-Lang Kalahari Expedition
H_BOT_129	FMNH_35741	Southern	BOT	1930	-19.1953	24.0070	Mababe Flats	Vernay-Lang Kalahari Expedition
H_BOT_130	FMNH_35742	Southern	BOT	1930	-19.1953	24.0070	Mababe Flats	Vernay-Lang Kalahari Expedition
H_BOT_131	FMNH_35743	Southern	BOT	1930	-19.7000	26.4000	Nata, 35 mi SW; N'kate	Vernay-Lang Kalahari Expedition
H_RSA_132	FMNH_38134	Southern	RSA	1928	-24.3697	30.6441	Transvaal, Pretoria Dist, Olifants R	H. Lang
H_BOT_133	FMNH_41405	Southern	BOT	1930	-19.1953	24.0070	Mababe Flats	Vernay-Lang Kalahari Expedition
H_MLI_134	FMNH_42129	Western	MLI	1920	14.3496	-3.6102	Mopti, Bandiagara	R. Boulton
H_KEN_135	FMNH_75608	Eastern	KEN	1935	0.5160	35.3234	Kenya	H. C. Pearson
H_KEN_136	FMNH_75609	Eastern	KEN	1935	0.5160	35.3234	Kenya	H. C. Pearson
H_BOT_137	FMNH_89926	Southern	BOT	1935	-23.0000	24.0000	Botswana	Vernay-Lang Kalahari Expedition
H_SDN_138	KBIN_469459	Western	SDN	1905	15.0000	30.0000	Sudan	
H_ZAM_139	KBIN_504512	Southern	ZAM	1905	-8.6606	29.8588	Mweru Wantipa - Zambia	
H_TAN_140	KU_105216	Eastern	TAN	1920	-2.9400	37.3400	Mt Kilomonjaro	Leasure F G
H_TAN_141	KU_105217	Eastern	TAN	1920	-2.9400	37.3400	Mt Kilomonjaro	Leasure F G
H_KEN_142	LACM_51295	Eastern	KEN	1922	-1.3547	36.8216	Nairobi	J. Dines
H_KEN_143	LACM_51296	Eastern	KEN	1922	-1.3547	36.8216	Nairobi	J. Dines
H_KEN_144	LACM_51297	Eastern	KEN	1922	-1.3547	36.8216	Nairobi	J. Dines
H_TAN_145	MVZ_96804	Eastern	KEN	1929	-1.3547	36.8216	Tanganyika District Safari Service from town of Nairobi	Ward C. Russell, A. F. Lieuranse
H_ANG_146	RMNH_45281	Southern	ANG	1887	-15.1996	12.1578	Mossamedes, Angola	
H_NRT_147	RMNH_45282	Northern	NRT	1823	35.0410	0.2941	Berberleeuw (Barbary: Dutch)	
H_MOZ_148	RMNH_45284	Southern	MOZ	1929	-18.0000	35.0000	Caia omgeving, Mozambique	
H_MOZ_149	RMNH_45285	Southern	MOZ	1929	-18.0000	35.0000	Caia omgeving, Mozambique	
H_NAM_150	S_581971	Southern	NAM	1856	-23.0679	14.5063	Namibia, Erongo, Walvis Bay	
H_NRT_151	S_585287	Northern	NRT	1831	35.0410	0.2941	Barbariet (Barbary: Swedish)	
H_DRC_152	S_595059	Western	DRC	1921	-0.7556	29.3318	Congo, Ruindi Plains S. of Lake Edward	
H_TAN_153	YPM_2057	Eastern	TAN	1928	-3.0000	35.0000	Tanganyika Territory: Serengeti Plains	S. Clark
H_KEN_154	YPM_3189	Eastern	KEN	1931	-1.0000	35.0000	Rift Valley Province, Masai Reservation, Amorro River	J.C. Rathborne
H_KEN_155	YPM_3217	Eastern	KEN	1931	-1.0000	35.0000	Rift Valley Province, Masai Reservation, Amorro River	J.C. Rathborne
H_KEN_156	YPM_3218	Eastern	KEN	1931	-1.0000	35.0000	Rift Valley Province, Masai Reservation, Amorro River	J.C. Rathborne
H_KEN_157	YPM_5251	Eastern	KEN	*1922*	-1.0000	34.0000	East Africa	Morden African Expedition
H_CPT_158	YPM_6943	Captive	CAP				(menagerie)	G.B. Grinnell
H_UNK_159	YPM_6952	Unknown	UNK	*1917*				H.W. Boyd
H_KEN_160	YPM_9570	Eastern	KEN	1931	-1.0000	35.0000	Rift Valley Province, Masai Reservation, Amorro River	J.C. Rathborne
H_RSA_161	ZMA_11753	Southern	RSA	1890	-24.3697	30.6441	between Pretoria and Lourenco Marques, South Africa	
H_CAR_162	ZMA_996	Western	CAR	1905	10.2910	22.7837	Birao, Central African Republic	

B.1.e: HISTORICAL LIONS – RESULTS (2 PAGES)

		Datasets		Original	Extraction	NGS Coverage			mtDNA Sequence Variation							STR % Amplification		
Project ID	Museum ID	nDNA	mtDNA	Material	Protocol	ng/uL	DNA	mtDNA	% Called	%Missing	REF	ALT	?	Haplotype	Clade	14 Loci	8 Loci	9 Loci
H_BOT_001	AMNH_119594	X	X	Turbinates	Bone	17.6	2.8	27.2	96.2%	3.8%	1692	51	69	Hap_72	S	64%	82%	82%
H_BOT_002	AMNH_119595	X	X	Tooth	Bone	12.6	2.2	92.7	99.6%	0.4%	1724	80	8	Hap_83	S	100%	100%	100%
H_COG_003	AMNH_119870	X	X	Tooth	Bone	3.1	2.1	128.6	99.7%	0.3%	1724	82	6	Hap_77	S	93%	100%	100%
H_GAB_004	AMNH_119871	X	X	Bone	Bone	13.2	1.8	103.0	99.7%	0.3%	1724	82	6	Hap_77	S	100%	100%	100%
H_CPT_005	AMNH_13904			Bone	Bone	13.7	3.4	8.8	75.4%	24.6%	1357	10	445			57%	73%	73%
H_CPT_006	AMNH_13998			Bone	Bone	18.9	2.4	262.8	99.6%	0.4%	1785	20	7			100%	100%	100%
H_CPT_007	AMNH_14027			Turbinates	Bone	11.8	2.9	300.8	99.6%	0.4%	1784	20	8			79%	91%	91%
H_CPT_008	AMNH_14028			Turbinates	Bone	8.7	3.0	665.2	99.6%	0.4%	1708	96	8			43%	45%	45%
H_CPT_009	AMNH_14034			Turbinates	Bone	18.0	3.0	2.6	5.9%	94.1%	107	0	1705			100%	100%	100%
H_ZIM_010	AMNH_161011	X	X	Bone	Bone	9.5	2.2	128.7	99.3%	0.7%	1724	76	12	Hap_76	S	100%	100%	100%
H_MWI_011	AMNH_161732	X	X	Tooth	Bone	1.4	2.4	85.3	99.3%	0.7%	1777	22	13	Hap_42	M	86%	100%	100%
H_COG_012	AMNH_17274		X	Tooth	Bone	5.3	2.3	21.8	98.5%	1.5%	1763	22	27	Hap_40	M	64%	82%	82%
H_COG_013	AMNH_17275		X	Bone	Bone	14.7	2.9	103.5	99.5%	0.5%	1781	22	9	Hap_41	M	100%	100%	100%
H_NAM_014	AMNH_19181	X	X	Tooth	Bone	5.9	2.0	54.4	99.0%	1.0%	1775	19	18	Hap_49	M	79%	91%	91%
H_CPT_015	AMNH_24249			Tooth	Bone	3.3	2.1	172.6	99.7%	0.3%	1707	99	6			86%	91%	91%
H_KEN_016	AMNH_27769	X	X	Hide	Bone	84.1	2.5	377.6	99.6%	0.4%	1725	80	7	Hap_32	E	79%	82%	82%
H_RSA_017	AMNH_28151	X		Turbinates	Bone	5.0	3.1	7.5	39.2%	60.8%	700	11	1101			71%	82%	82%
H_KEN_018	AMNH_30240	X		Hide	Bone	8.9	5.4	2.0	0.1%	99.9%	2	0	1810			86%	91%	91%
H_KEN_019	AMNH_30241	X	X	Tooth	Bone	3.3	2.3	174.0	99.5%	0.5%	1782	21	9	Hap_65	M	71%	82%	82%
H_KEN_020	AMNH_30242	X	X	Tooth	Bone	6.2	2.5	75.5	98.8%	1.2%	1769	21	22	Hap_65	M	93%	100%	100%
H_KEN_021	AMNH_30243	X	X	Tooth	Bone	11.0	2.0	47.3	98.9%	1.1%	1770	22	20	Hap_66	M	93%	100%	100%
H_KEN_022	AMNH_30244	X	X	Tooth	Bone	7.1	3.1	240.2	99.6%	0.4%	1782	22	8	Hap_66	M	100%	100%	100%
H_KEN_023	AMNH_30245	X	X	Tooth	Bone	10.8	2.2	96.2	99.0%	1.0%	1772	22	18	Hap_66	M	93%	100%	100%
H_KEN_024	AMNH_30246	X	X	Tooth	Bone	1.5	3.7	202.8	98.6%	1.4%	1765	21	26	Hap_64	M	71%	82%	82%
H_KEN_025	AMNH_30247	X	X	Tooth	Bone	5.6	2.4	71.2	98.8%	1.2%	1769	22	21	Hap_66	M	79%	100%	100%
H_KEN_026	AMNH_30248	X	X	Tooth	Bone	21.3	2.1	26.6	98.7%	1.3%	1765	23	24	Hap_43	M	100%	100%	100%
H_CPT_027	AMNH_35472			Bone	Bone	51.5	3.8	3.1	11.5%	88.5%	205	3	1604			93%	100%	100%
H_KEN_028	AMNH_36420	X	X	Tooth	Bone	13.1	2.1	169.6	99.4%	0.6%	1780	21	11	Hap_47	M	100%	100%	100%
H_KEN_029	AMNH_36421	X	X	Tooth	Bone	1.0	3.5	1482.9	99.6%	0.4%	1783	21	8	Hap_47	M	71%	82%	82%
H_CPT_030	AMNH_403			Bone	Bone	7.3	3.4	2.5	2.9%	97.1%	53	0	1759			64%	64%	64%
H_DRC_031	AMNH_52070	X	X	Tooth	Bone	7.5	3.8	372.9	99.5%	0.5%	1709	94	9	Hap_13	W	93%	100%	100%
H_DRC_032	AMNH_52071	X		Turbinates	Bone	149.0	4.3	10.1	81.8%	18.2%	1425	58	329			93%	100%	100%
H_DRC_033	AMNH_52072	X	X	Bone	Bone	39.8	2.5	12.3	88.1%	11.9%	1536	61	215	Hap_7	W	93%	100%	100%
H_DRC_034	AMNH_52073	X	X	Bone	Bone	2.5	3.8	65.1	96.2%	3.8%	1723	21	68	Hap_51	M	93%	100%	100%
H_DRC_035	AMNH_52074	X	X	Tooth	Bone	2.0	2.6	35.6	98.7%	1.3%	1701	87	24	Hap_11	W	71%	82%	82%
H_DRC_036	AMNH_52075	X	X	Tooth	Bone	2.9	2.6	217.8	99.6%	0.4%	1710	95	7	Hap_15	W	57%	64%	64%
H_DRC_037	AMNH_52076	X		Turbinates	Bone	3.1	3.3	4.8	38.9%	61.1%	687	18	1107			64%	73%	73%
H_DRC_038	AMNH_52077	X	X	Tooth	Bone	1.6	9.6	18.1	93.5%	6.5%	1628	66	118	Hap_9	W	93%	100%	100%
H_DRC_039	AMNH_52078	X	X	Bone	Bone	11.5	2.7	142.3	99.1%	0.9%	1700	96	16	Hap_16	W	100%	100%	100%
H_DRC_040	AMNH_52079	X	X	Tooth	Bone	2.4	2.2	297.7	99.6%	0.4%	1711	94	7	Hap_12	W	93%	100%	100%
H_DRC_041	AMNH_52080	X		Tooth	Bone	2.7	2.8	5.0	42.4%	57.6%	740	29	1043			71%	82%	82%
H_DRC_042	AMNH_52081	X	X	Bone	Bone	25.0	2.5	158.3	99.6%	0.4%	1710	95	7	Hap_15	W	100%	100%	100%
H_DRC_043	AMNH_52082	X	X	Tooth	Bone	7.8	3.5	326.9	99.7%	0.3%	1711	96	5	Hap_14	W	79%	91%	91%
H_KEN_044	AMNH_54370	X	X	Bone	Bone	28.0	2.0	100.8	99.4%	0.6%	1718	83	11	Hap_29	E	100%	100%	100%
H_KEN_045	AMNH_54371	X	X	Bone	Bone	11.0	2.2	111.5	99.1%	0.9%	1714	82	16	Hap_30	E	100%	100%	100%
H_KEN_046	AMNH_54372	X	X	Bone	Bone	10.4	3.3	17.6	96.0%	4.0%	1680	60	72	Hap_24	E	100%	100%	100%
H_KEN_047	AMNH_54393	X		Bone	Bone	65.9	4.0	2.7	7.8%	92.2%	135	7	1670			93%	100%	100%
H_KEN_048	AMNH_54394	X		Bone	Bone	34.8	3.7	25.2	45.0%	55.0%	607	209	996			100%	100%	100%
H_KEN_049	AMNH_54395	X		Bone	Bone	67.1	2.9	7.9	30.7%	69.3%	401	155	1256			93%	100%	100%
H_GIR_050	AMNH_54995	X	X	Dried Tissue	Bone	1.9	2.9	255.2	97.9%	2.1%	1685	89	38	Hap_1	W-N	79%	91%	91%
H_GIR_051	AMNH_54996	X	X	Dried Tissue	Bone	8.2	2.2	957.3	99.4%	0.6%	1704	98	10	Hap_3	W-N	79%	91%	91%
H_CPT_052	AMNH_6260			Bone	Bone	22.0	2.0	69.3	99.3%	0.7%	1724	76	12			100%	100%	100%
H_CPT_053	AMNH_6282			Turbinates	Bone	16.5	2.0	16.9	91.5%	8.5%	1601	57	154			100%	100%	100%
H_GIR_054	AMNH_63955	X	X	Tooth	Bone	8.1	2.6	231.9	99.4%	0.6%	1705	97	10	Hap_2	W-N	100%	100%	100%
H_CPT_055	AMNH_65			Tooth	Bone	12.5	1.8	49.0	99.3%	0.7%	1780	19	13			93%	100%	100%
H_CPT_056	AMNH_70171			Bone	Bone	12.2	5.7	55.5	98.7%	1.3%	1697	91	24			86%	91%	91%
H_KEN_057	AMNH_70347	X	X	Bone	Bone	17.7	2.4	93.1	99.4%	0.6%	1704	97	11	Hap_18	W	93%	100%	100%
H_ANG_058	AMNH_80609	X		Bone	Bone	72.5	2.0	5.1	44.6%	55.4%	795	14	1003			100%	100%	100%
H_RSA_059	AMNH_81836	X	X	Tooth	Bone	7.3	2.2	166.0	99.6%	0.4%	1720	84	8	Hap_69	S	93%	100%	100%
H_RSA_060	AMNH_81837	X	X	Tooth	Bone	5.3	2.4	400.0	99.7%	0.3%	1726	80	6	Hap_68	S	93%	91%	91%
H_RSA_061	AMNH_81839	X	X	Tooth	Bone	1.4	2.0	54.4	99.1%	0.9%	1718	77	17	Hap_67	S	79%	91%	91%
H_RSA_062	AMNH_81840	X	X	Tooth	Bone	283.0	2.5	305.2	99.7%	0.3%	1726	80	6	Hap_68	S	100%	100%	100%
H_RSA_063	AMNH_81841	X	X	Tooth	Bone	4.2	2.8	430.6	99.8%	0.2%	1788	20	4	Hap_50	M	79%	91%	91%
H_RSA_064	AMNH_81842	X	X	Tooth	Bone	8.0	2.1	1703.9	99.9%	0.1%	1790	20	2	Hap_50	M	93%	100%	100%
H_RSA_065	AMNH_81843	X	X	Tooth	Bone	18.1	1.9	202.9	99.7%	0.3%	1787	20	5	Hap_50	M	100%	100%	100%
H_RSA_066	AMNH_81844	X	X	Bone	Bone	6.4	2.7	161.1	99.1%	0.9%	1712	84	16	Hap_70	S	93%	100%	100%
H_CAR_067	AMNH_83410	X	X	Tooth	Bone	19.6	1.9	174.3	99.6%	0.4%	1709	96	7	Hap_14	W	100%	100%	100%
H_CPT_068	AMNH_8355			Turbinates	Bone	27.3	4.8	4.9	38.0%	62.0%	669	20	1123			86%	100%	100%
H_BOT_069	AMNH_83617	X	X	Turbinates	Bone	3.5	3.6	15.7	95.8%	4.2%	1671	64	77	Hap_73	S	93%	100%	100%
H_BOT_070	AMNH_83618	X	X	Turbinates	Bone	8.0	2.0	73.9	99.1%	0.9%	1714	82	16	Hap_88	S	100%	100%	100%
H_BOT_071	AMNH_83619	X	X	Tooth	Bone	29.1	2.2	108.9	99.7%	0.3%	1726	80	6	Hap_78	S	100%	100%	100%
H_BOT_072	AMNH_83620	X	X	Tooth	Bone	1.4	1.9	17.0	90.1%	9.9%	1604	28	180	Hap_35	S	43%	55%	55%
H_BOT_073	AMNH_83621	X	X	Turbinates	Bone	15.4	1.9	51.3	98.8%	1.2%	1718	73	21	Hap_80	S	100%	100%	100%
H_BOT_074	AMNH_83622	X	X	Bone	Bone	8.7	2.2	81.5	99.0%	1.0%	1717	76	19	Hap_81	S	100%	100%	100%
H_BOT_075	AMNH_83623	X	X	Bone	Bone	6.9	2.3	74.8	99.1%	0.9%	1715	80	17	Hap_87	S	100%	100%	100%
H_BOT_076	AMNH_83624	X	X	Turbinates	Bone	20.9	2.0	56.4	98.8%	1.2%	1713	78	21	Hap_85	S	100%	100%	100%
H_BOT_077	AMNH_83625	X	X	Tooth	Bone	14.4	2.1	138.2	99.7%	0.3%	1724	83	5	Hap_89	S	86%	91%	91%
H_CPT_078	AMNH_8364			Tooth	Bone	17.9	5.1	10.7	81.4%	18.6%	1428	47	337			79%	82%	82%
H_TAN_079	AMNH_85140	X	X	Dried Tissue	Bone	8.8	6.2	115.5	99.1%	0.9%	1774	21	17	Hap_65	M	86%	100%	100%
H_TAN_080	AMNH_85141-L	X	X	Tooth	Bone	3.3	1.9	46.7	99.1%	0.9%	1774	22	16	Hap_66	M	93%	100%	100%
H_TAN_081	AMNH_85141-N	X	X	Bone	Bone	12.8	2.2	26.7	96.4%	3.6%	1727	19	66	Hap_56	M	100%	100%	100%

B.1.e: HISTORICAL LIONS – RESULTS (PAGE 2 OF 2)

		Datasets		Original	Extraction	NGS Coverage			mtDNA Sequence Variation							STR % Amplification		
Project ID	Museum ID	nDNA	mtDNA	Material	Protocol	ng/uL	DNA	mtDNA	% Called	%Missing	REF	ALT	?	Haplotype	Clade	14 Loci	8 Loci	9 Loci
H_TAN_082	AMNH_85142-L	X		Tooth	Bone	5.1	2.3	2.8	11.2%	88.8%	202	1	1609			71%	82%	82%
H_TAN_083	AMNH_85142-N	X	X	Bone	Bone	10.0	2.2	30.6	97.8%	2.2%	1751	21	40	Hap_65	M	100%	100%	100%
H_TAN_084	AMNH_85143	X	X	Bone	Bone	7.1	2.3	55.6	98.8%	1.2%	1769	22	21	Hap_66	M	100%	100%	100%
H_TAN_085	AMNH_85144	X	X	Bone	Bone	5.5	2.4	17.7	93.3%	6.7%	1674	16	122	Hap_54	M	86%	100%	100%
H_TAN_086	AMNH_85145	X	X	Tooth	Bone	1.8	1.8	22.1	98.3%	1.7%	1762	20	30	Hap_60	M	86%	91%	91%
H_TAN_087	AMNH_85146	X	X	Bone	Bone	142.0	1.9	94.9	99.4%	0.6%	1778	23	11	Hap_59	M	100%	100%	100%
H_TAN_088	AMNH_85147	X	X	Dried Tissue	Bone	9.5	2.1	327.9	99.6%	0.4%	1782	22	8	Hap_66	M	57%	64%	64%
H_TAN_089	AMNH_85148	X	X	Bone	Bone	6.6	2.9	159.1	98.8%	1.2%	1769	21	22	Hap_64	M	100%	100%	100%
H_TAN_090	AMNH_85149	X	X	Bone	Bone	9.3	2.2	24.6	94.6%	5.4%	1697	17	98	Hap_45	M	100%	100%	100%
H_KEN_091	AMNH_88632	X	X	Tooth	Bone	2.8	2.0	51.5	99.1%	0.9%	1717	78	17	Hap_27	E	100%	100%	100%
H_KEN_092	AMNH_88633	X	X	Tooth	Bone	2.3	1.8	29.9	97.5%	2.5%	1700	66	46	Hap_25	E	64%	82%	82%
H_KEN_093	AMNH_88634	X		Tooth	Bone	9.2	1.7	7.8	75.6%	24.4%	1353	17	442			86%	91%	91%
H_KEN_094	AMNH_88635	X	X	Tooth	Bone	4.4	2.6	222.5	99.3%	0.7%	1718	82	12	Hap_31	E	93%	100%	100%
H_KEN_095	AMNH_88636	X	X	Tooth	Bone	11.5	2.1	41.5	98.9%	1.1%	1711	81	20	Hap_28	E	100%	100%	100%
H_KEN_096	AMNH_88637	X	X	Tooth	Bone	3.8	2.1	64.0	99.3%	0.7%	1717	82	13	Hap_26	E	100%	100%	100%
H_CPT_097	CM_1461			Tooth	Bone	5.0	2.9	14.7	92.2%	7.8%	1598	72	142			64%	82%	82%
H_CPT_098	CM_1564			Dried Tissue	DNeasy	3.5	2.4	91.1	98.3%	1.7%	1696	86	30			93%	100%	100%
H_CPT_099	CM_1565			Dried Tissue	DNeasy	3.4	2.1	59.0	99.0%	1.0%	1703	90	19			86%	91%	91%
H_CPT_100	CM_1825			Bone	Bone	10.5	5.0	17.4	94.3%	5.7%	1650	59	103			50%	64%	64%
H_UNK_101	CM_184			Tooth	Bone	1.6	1.9	9.6	76.3%	23.7%	1334	48	430			43%	55%	55%
H_UNK_102	CM_185			Dried Tissue	DNeasy	1.2	2.9	3.3	9.6%	90.4%	173	1	1638			36%	45%	45%
H_UNK_103	CM_31			Turbinates	Bone	15.1	4.8	2.3	3.1%	96.9%	56	0	1756			93%	100%	100%
H_ETH_104	CM_5868	X	X	Tooth	Bone	1.8	2.0	21.5	98.2%	1.8%	1759	20	33	Hap_46	M	57%	73%	73%
H_TAN_105	CM_5897	X	X	Bone	Bone	2.9	3.1	21.0	93.0%	7.0%	1668	18	126	Hap_44	M	86%	91%	91%
H_TAN_106	CM_5898	X	X	Dried Tissue	DNeasy	6.6	2.1	98.5	99.3%	0.7%	1779	21	12	Hap_65	M	71%	82%	82%
H_TAN_107	CM_5899	X	X	Turbinates	Bone	19.8	2.1	18.7	95.8%	4.2%	1715	20	77	Hap_58	M	100%	100%	100%
H_TAN_108	FMNH_127836	X	X	Dried Tissue	DNeasy	2.6	2.9	21.8	94.7%	5.3%	1698	18	96	Hap_53	M	64%	82%	82%
H_TAN_109	FMNH_127837	X		Dried Tissue	DNeasy	2.5	5.1	4.1	17.0%	83.0%	306	2	1504			50%	55%	55%
H_TAN_110	FMNH_127838	X		Dried Tissue	DNeasy	8.4	5.9	3.4	12.1%	87.9%	216	3	1593			71%	91%	91%
H_TAN_111	FMNH_127839	X	X	Dried Tissue	DNeasy	5.3	2.9	59.4	98.0%	2.0%	1755	20	37	Hap_62	M	43%	55%	55%
H_TAN_112	FMNH_127840	X		Dried Tissue	DNeasy	2.1	8.8	2.7	1.2%	98.8%	22	0	1790			36%	45%	45%
H_SOM_113	FMNH_1443	X	X	Dried Tissue	DNeasy	1.3	3.1	37.2	94.3%	5.7%	1627	81	104	Hap_10	W	57%	73%	73%
H_KEN_114	FMNH_20756	X		Dried Tissue	DNeasy	1.3	2.8	7.0	5.2%	94.8%	66	29	1717			64%	73%	73%
H_KEN_115	FMNH_20757	X	X	Dried Tissue	DNeasy	2.5	2.4	57.3	96.9%	3.1%	1734	21	57	Hap_63	M	100%	100%	100%
H_KEN_116	FMNH_20758	X	X	Dried Tissue	DNeasy	1.2	2.9	34.9	97.7%	2.3%	1751	20	41	Hap_62	M	57%	73%	73%
H_KEN_117	FMNH_20760	X	X	Dried Tissue	DNeasy	1.5	2.4	312.0	99.7%	0.3%	1784	22	6	Hap_66	M	71%	82%	82%
H_KEN_118	FMNH_20762	X	X	Dried Tissue	DNeasy	1.5	2.8	97.4	98.4%	1.6%	1762	21	29	Hap_61	M	86%	100%	100%
H_SDN_119	FMNH_30778	X		Dried Tissue	DNeasy	1.0	2.4	2.1	1.2%	98.8%	21	0	1791			64%	73%	73%
H_GIR_120	FMNH_31121	X	X	Dried Tissue	DNeasy	2.1	2.6	287.3	99.4%	0.6%	1703	98	11	Hap_4	W-N	100%	100%	100%
H_TAN_121	FMNH_33479	X		Dried Tissue	Bone	3.7	3.0	12.6	85.8%	14.2%	1544	10	258			7%	9%	9%
H_TAN_122	FMNH_33480	X		Dried Tissue	DNeasy	3.6	3.8	5.2	45.8%	54.2%	820	9	983			71%	91%	91%
H_TAN_123	FMNH_35131	X	X	Dried Tissue	DNeasy	1.9	3.6	182.5	99.6%	0.4%	1783	22	7	Hap_66	M	50%	64%	64%
H_TAN_124	FMNH_35132	X	X	Dried Tissue	DNeasy	6.5	3.5	1459.0	99.7%	0.3%	1784	22	6	Hap_66	M	29%	36%	36%
H_TAN_125	FMNH_35133	X	X	Dried Tissue	DNeasy	12.1	1.8	140.0	99.7%	0.3%	1784	22	6	Hap_66	M	100%	100%	100%
H_TAN_126	FMNH_35134	X	X	Dried Tissue	DNeasy	6.2	2.2	562.5	99.9%	0.1%	1788	22	2	Hap_66	M	100%	100%	100%
H_BOT_127	FMNH_35739	X	X	Dried Tissue	DNeasy	1.4	3.3	204.2	99.2%	0.8%	1777	20	15	Hap_50	M	86%	91%	91%
H_BOT_128	FMNH_35740	X		Dried Tissue	DNeasy	2.3	7.0	4.8	34.0%	66.0%	616	0	1196			57%	64%	64%
H_BOT_129	FMNH_35741	X	X	Dried Tissue	DNeasy	4.3	2.3	239.7	99.7%	0.3%	1727	79	6	Hap_79	S	86%	100%	100%
H_BOT_130	FMNH_35742	X		Dried Tissue	DNeasy	6.8	2.2	10.9	81.5%	18.5%	1430	47	335			71%	91%	91%
H_BOT_131	FMNH_35743	X	X	Dried Tissue	DNeasy	3.2	3.1	369.7	99.2%	0.8%	1718	79	15	Hap_79	S	57%	64%	64%
H_RSA_132	FMNH_38134	X	X	Dried Tissue	DNeasy	12.0	2.1	82.4	99.3%	0.7%	1723	76	13	Hap_75	S	100%	100%	100%
H_BOT_133	FMNH_41405	X	X	Dried Tissue	DNeasy	4.5	2.5	262.1	99.6%	0.4%	1725	80	7	Hap_82	S	100%	100%	100%
H_MLI_134	FMNH_42129	X		Dried Tissue	DNeasy	1.7	2.5	11.2	74.5%	25.5%	1321	29	462			71%	82%	82%
H_KEN_135	FMNH_75608	X		Dried Tissue	DNeasy	28.4	5.2	1.9	0.6%	99.4%	10	0	1802			21%	27%	27%
H_KEN_136	FMNH_75609	X		Dried Tissue	DNeasy	5.3	8.5	9.5	81.6%	18.4%	1466	12	334			50%	55%	55%
H_BOT_137	FMNH_89926	X	X	Dried Tissue	DNeasy	3.3	2.7	231.3	99.6%	0.4%	1724	81	7	Hap_84	S	100%	100%	100%
H_SDN_138	KBIN_469459	X		Skull	***											14%	18%	18%
H_ZAM_139	KBIN_504512	X		Skull	***											86%	91%	91%
H_TAN_140	KU_105216	X	X	Bone	Bone	1.0	1.9	62.4	98.3%	1.7%	1767	15	30	Hap_52	M	79%	82%	82%
H_TAN_141	KU_105217	X	X	Bone	Bone	125.0	2.6	125.6	99.4%	0.6%	1781	21	10	Hap_65	M	100%	100%	100%
H_KEN_142	LACM_51295	X	X	Bone	Bone	97.1	2.1	11.8	88.1%	11.9%	1578	18	216	Hap_55	M	93%	100%	100%
H_KEN_143	LACM_51296	X		Bone	Bone	12.6	2.1	9.5	80.9%	19.1%	1452	14	346			100%	100%	100%
H_KEN_144	LACM_51297	X		Bone	DNeasy	1.3	3.5	2.1	1.2%	98.8%	21	0	1791			36%	36%	36%
H_TAN_145	MVZ_96804	X	X	Turbinates	Bone	3.2	3.8	14.7	95.6%	4.4%	1711	21	80	Hap_48	M	64%	73%	73%
H_ANG_146	RMNH_45281	X		Skull	***											71%	82%	82%
H_NRT_147	RMNH_45282	X		Hide	***											50%	55%	55%
H_MOZ_148	RMNH_45284	X		Skull	***											36%	45%	45%
H_MOZ_149	RMNH_45285	X		Skull	***											57%	64%	64%
H_NAM_150	S_581971	X		Skull	***											93%	100%	100%
H_NRT_151	S_585287	X		Skull	***											43%	45%	45%
H_DRC_152	S_595059	X		Skull	***											64%	73%	73%
H_TAN_153	YPM_2057	X	X	Hide	Bone	1.3	2.7	228.0	97.6%	2.4%	1748	21	43	Hap_57	M	71%	82%	82%
H_KEN_154	YPM_3189	X	X	Hide	Bone	4.5	2.5	372.5	99.2%	0.8%	1775	22	15	Hap_66	M	57%	64%	64%
H_KEN_155	YPM_3217	X	X	Hide	Bone	10.6	2.3	585.0	99.4%	0.6%	1719	82	11	Hap_33	E	93%	100%	100%
H_KEN_156	YPM_3218	X	X	Hide	Bone	4.4	2.2	185.1	99.1%	0.9%	1778	17	17	Hap_36	M	93%	100%	100%
H_KEN_157	YPM_5251	X	X	Dried Tissue	Bone		2.3	321.9	99.4%	0.6%	1720	81	11	Hap_34	E	79%	91%	91%
H_CPT_158	YPM_6943			Bone	Bone	19.7	2.5	30.4	92.9%	7.1%	1615	69	128			100%	100%	100%
H_UNK_159	YPM_6952			Bone	Bone	18.9	2.5	3.0	10.7%	89.3%	191	2	1619			86%	91%	91%
H_KEN_160	YPM_9570	X		Dried Tissue	DNeasy	14.3	1.9	11.5	89.1%	10.9%	1601	13	198			100%	100%	100%
H_RSA_161	ZMA_11753	X		Skull	***											50%	55%	55%
H_CAR_162	ZMA_996	X		Skull	***											86%	91%	91%

B.2.a: ZAMBIAN STR ALLELE CALLS (11 PAGES)

TAMUID	Leo006	Leo008	Leo031	Leo045	Leo077	Leo085	Leo098	Leo126	Leo224	Leo230	Leo247	Leo281	Leo391	Leo506
2011000254	94 104	113 131	192 196	80 80	98 102	80 94	102 102	125 129	86 86	80 88	122 122	211 229	194 198	193 197
2011000255	108 122	113 131	192 196	80 80	104 104	80 94	106 106	105 127	86 86	0 0	116 128	213 247	174 174	191 191
2011000256	94 122	113 131	188 196	80 80	98 104	74 80	104 106	125 129	78 94	0 0	128 128	247 247	174 174	171 193
2011000257	94 108	113 129	196 196	80 80	102 110	80 94	104 104	127 127	86 86	0 0	116 128	213 213	174 174	171 193
2011000258	104 108	111 111	196 196	80 94	102 104	80 80	102 102	127 145	86 92	80 90	114 122	213 223	174 178	191 191
2011000259	122 122	111 131	196 196	80 100	104 112	80 80	102 106	125 127	86 86	88 88	128 132	217 245	174 178	173 193
2011000260	108 124	113 113	196 196	80 96	98 104	76 80	102 102	125 125	86 92	76 76	126 126	213 213	174 174	173 173
2011000261	82 82	111 113	196 196	80 80	98 110	76 80	92 102	125 127	86 92	76 82	122 126	217 245	174 178	173 173
2011000262	104 122	111 131	196 196	80 80	104 110	80 80	92 106	125 127	78 92	76 88	122 122	245 245	174 174	173 195
2011000263	108 122	111 131	196 196	80 102	98 104	76 80	102 104	127 127	78 92	80 88	122 126	213 231	170 174	173 195
2011000264	122 124	111 113	196 196	80 80	98 102	80 80	92 92	105 131	96 96	76 88	126 126	213 233	174 174	193 195
2011000265	94 104	113 127	196 196	80 80	102 104	74 80	102 104	125 125	92 92	0 0	114 128	213 213	174 174	171 193
2011000267	110 122	127 131	196 196	80 80	102 104	80 86	92 104	127 127	78 86	0 0	114 126	213 229	190 190	195 195
2011000268	108 108	113 129	188 196	80 80	102 104	74 80	102 108	105 125	86 92	0 0	122 128	213 213	174 174	173 173
2011000269	104 124	113 127	196 196	80 94	102 110	80 80	102 102	125 127	78 78	88 88	124 128	213 213	174 174	193 195
2011000270	82 124	113 113	186 196	80 80	98 102	74 80	92 104	105 127	94 96	0 0	128 132	213 233	190 198	193 195
2011000271	100 124	113 127	188 196	80 80	104 104	76 76	104 108	125 125	78 78	80 88	116 128	213 213	166 198	191 195
2011000272	108 122	113 127	196 196	80 80	102 104	74 76	102 102	125 125	78 92	76 80	122 128	213 245	174 194	173 199
2011000273	94 108	111 111	188 196	80 94	98 104	76 94	102 102	127 127	78 86	0 0	128 132	217 217	174 198	191 195
2011000274	122 124	111 113	196 196	80 100	108 112	70 76	106 108	125 125	78 78	0 0	114 116	213 249	174 174	191 195
2011000275	108 124	111 113	188 196	80 80	98 102	76 80	102 102	125 131	86 96	76 76	116 126	213 213	174 174	173 193
2011000276	104 104	111 111	196 196	80 80	104 112	80 80	92 104	125 127	92 96	88 88	116 122	213 251	190 194	173 195
2011000277	108 122	111 127	188 196	80 80	104 106	80 80	102 106	105 125	78 86	76 88	114 132	213 213	174 174	193 193
2011000278	104 124	111 127	196 196	80 80	98 104	80 80	104 106	127 131	78 92	92 92	114 122	213 249	174 194	173 195
2011000279	94 108	113 127	196 196	80 80	98 104	74 80	104 106	125 125	86 92	0 0	114 126	213 217	174 174	173 195
2011000280	108 124	111 111	196 196	80 94	104 104	74 80	102 102	127 127	86 94	0 0	114 128	213 217	178 186	193 193
2011000281	104 104	127 131	196 196	80 80	104 108	76 80	104 104	143 143	78 86	0 0	116 128	217 223	174 174	193 195
2011000282	122 124	113 125	188 200	80 80	98 106	80 80	102 102	125 127	86 92	76 76	126 132	213 213	174 178	173 191
2011000283	124 124	111 113	196 196	80 80	102 104	70 76	104 108	125 125	78 78	0 0	126 132	213 213	194 198	191 193
2011000284	124 124	113 113	188 196	80 80	102 104	76 80	104 108	125 125	78 78	0 0	126 126	213 213	174 198	191 193
2011000285	108 124	111 113	196 196	80 102	104 104	80 80	106 106	127 131	78 86	76 88	128 128	213 217	182 190	171 191
2011000286	108 124	111 127	196 196	80 80	98 112	80 80	102 102	125 125	78 92	88 88	126 128	213 217	174 182	171 191
2011000287	94 130	113 131	196 196	80 80	104 108	80 94	102 104	125 127	86 92	92 92	122 126	217 223	174 194	173 197
2011000288	104 122	127 131	196 196	80 94	104 104	76 80	102 102	127 129	92 92	76 76	114 122	229 233	182 186	171 171
2011000290	122 124	111 127	196 196	80 94	102 104	76 92	104 106	105 127	78 78	0 0	128 128	213 225	174 190	181 191
2011000291	104 124	129 133	196 196	80 94	102 102	80 92	104 108	105 145	78 86	88 88	114 128	213 223	174 174	171 193
2011000293	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0
2011000295	104 128	111 113	196 196	80 80	104 104	74 80	102 106	105 127	78 92	0 0	116 128	213 233	174 174	191 191

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TAMUID	Leo006	Leo008	Leo031	Leo045	Leo077	Leo085	Leo098	Leo126	Leo224	Leo230	Leo247	Leo281	Leo391	Leo506
2011000296	104 108	111 111	188 196	94 94	104 104	80 80	102 102	127 129	86 94	76 90	114 114	213 229	174 178	191 193
2011000297	124 124	113 127	196 196	80 80	98 104	76 94	100 102	125 129	86 86	80 80	114 128	213 213	174 190	193 195
2011000298	124 124	111 111	196 196	80 100	98 102	80 94	102 102	127 127	86 86	0 0	114 116	213 223	178 194	191 193
2011000299	108 108	111 111	188 196	80 100	104 108	74 80	92 102	125 125	78 86	0 0	126 126	213 217	174 198	195 195
2011000300	108 122	111 113	186 196	80 80	98 106	80 80	102 106	125 127	88 92	76 76	122 128	213 213	174 198	173 195
2011000301	124 124	111 113	188 196	80 94	98 102	80 94	102 106	125 125	78 92	76 88	126 128	213 213	174 178	193 193
2011000302	104 108	111 125	188 196	80 80	102 104	80 80	102 102	125 135	86 92	78 92	114 116	229 251	174 178	191 195
2011000303	122 124	113 125	186 196	80 80	102 104	76 94	102 106	129 135	78 86	88 88	114 114	213 213	174 190	191 195
2011000304	82 82	111 111	196 196	80 80	98 112	80 80	102 106	125 129	86 86	80 80	116 126	213 231	174 174	193 193
2011000305	104 108	111 125	196 196	80 80	102 104	80 80	102 102	125 127	86 92	76 88	114 126	213 229	178 198	171 195
2011000306	94 124	111 113	192 196	94 102	102 108	80 80	104 106	105 125	78 80	82 90	126 128	213 213	174 198	191 195
2011000307	108 124	111 129	196 196	94 94	104 104	76 80	106 106	125 125	78 78	90 90	124 128	213 217	174 174	193 195
2011000308	94 96	113 113	188 196	80 80	106 106	80 80	104 104	125 125	78 86	76 76	126 128	213 245	174 174	171 193
2011000309	96 104	113 131	196 196	94 94	98 106	74 86	102 104	105 129	86 86	0 0	122 128	213 247	174 186	195 197
2011000310	96 130	131 131	196 196	80 80	98 104	74 76	102 108	127 129	78 78	76 80	114 128	213 213	182 186	171 187
2011000311	94 108	111 111	196 196	94 94	104 108	76 80	100 102	105 127	78 86	0 0	128 128	213 213	170 198	173 199
2011000312	108 124	113 113	188 196	94 96	104 112	74 74	102 104	125 125	78 92	80 88	126 126	213 243	174 178	195 199
2011000313	94 124	129 131	196 196	80 80	104 108	76 80	102 108	125 127	78 86	84 90	114 128	217 245	174 186	171 193
2011000314	94 124	113 113	196 196	80 102	104 108	80 80	104 106	105 125	78 80	88 94	122 128	213 217	174 174	191 193
2011000315	122 124	111 129	196 196	80 94	102 104	80 80	106 106	125 125	78 78	80 90	124 128	213 217	174 174	191 195
2011000316	94 104	111 127	186 196	80 80	102 104	92 92	104 106	129 129	78 94	0 0	114 114	213 213	174 198	191 193
2011000317	82 124	113 113	188 196	80 94	98 104	76 94	102 102	105 129	86 86	0 0	124 132	217 245	174 174	193 193
2011000318	104 128	111 111	196 196	80 80	98 106	80 94	106 106	105 145	86 86	76 88	122 132	213 213	178 178	173 193
2011000319	104 124	111 127	196 196	80 94	104 104	74 80	102 106	127 129	78 92	0 0	114 114	213 229	174 186	193 193
2011000320	108 108	111 113	194 196	80 80	108 112	76 80	102 104	125 125	78 92	80 88	128 128	213 229	174 190	191 193
2011000321	104 128	111 111	186 196	80 80	98 106	80 94	102 106	105 127	86 86	88 88	122 122	213 213	174 190	173 195
2011000322	108 108	111 111	196 196	80 94	98 106	80 94	102 106	105 127	78 86	76 76	124 132	213 217	174 178	193 195
2011000323	82 124	111 125	188 196	80 94	98 106	80 94	102 106	105 127	86 92	76 76	122 132	213 245	174 178	191 195
2011000324	124 124	111 113	196 196	80 80	98 106	80 80	102 102	125 127	86 94	88 88	128 128	213 233	174 174	173 173
2011000325	122 122	113 123	192 196	80 94	102 104	74 94	104 104	105 129	78 94	76 80	116 126	213 233	190 194	191 191
2011000326	108 122	113 123	196 196	80 94	102 104	76 94	92 104	105 129	78 86	76 86	122 126	213 231	190 194	191 191
2011000327	94 108	127 131	188 192	80 80	104 110	76 80	104 104	105 129	78 86	80 80	122 136	247 247	174 194	171 171
2011000328	104 104	111 127	196 196	80 80	104 106	76 80	104 104	127 127	86 86	76 90	114 128	213 215	174 186	193 195
2011000329	122 122	113 131	196 196	80 80	102 110	74 80	102 102	125 129	78 90	76 80	122 122	211 229	174 174	171 197
2011000330	104 128	113 127	188 196	80 80	98 104	80 94	102 106	105 127	86 92	76 80	114 130	213 213	174 174	193 193
2011000331	104 128	111 127	186 196	80 80	106 112	80 94	106 106	105 145	86 92	88 88	122 122	213 213	178 190	191 195
2011000332	82 124	111 131	196 196	80 80	104 112	76 80	106 106	125 125	86 86	80 80	126 128	213 233	174 174	193 195
2011000333	108 124	111 113	196 196	80 80	102 104	76 80	102 104	127 127	86 92	82 88	114 128	213 213	186 198	171 191

B.2.a: ZAMBIAN STR ALLELE CALLS (PAGE 3 OF 11)

TAMUID	Leo006	Leo008	Leo031	Leo045	Leo077	Leo085	Leo098	Leo126	Leo224	Leo230	Leo247	Leo281	Leo391	Leo506
2011000334	124 124	111 127	188 194	80 80	104 104	74 76	104 106	125 125	78 78	0 0	114 126	213 253	174 198	191 195
2011000335	108 124	113 125	196 196	80 80	104 104	74 80	106 106	105 125	80 86	0 0	124 128	213 213	174 190	191 195
2011000336	108 124	111 113	188 196	100 100	104 104	80 80	102 102	127 127	86 86	76 90	114 128	213 213	194 194	173 195
2011000337	122 124	111 113	188 194	94 102	102 106	74 76	104 106	125 125	86 86	76 80	122 128	213 217	174 174	171 173
2011000338	124 124	111 127	196 196	80 94	102 106	76 80	102 102	125 127	78 78	90 90	114 126	213 229	190 198	193 195
2011000339	108 124	111 111	188 196	80 94	106 108	80 80	102 106	125 125	78 86	88 88	116 132	213 251	182 186	193 195
2011000340	96 104	111 113	196 196	80 80	104 108	80 86	104 106	127 131	86 94	0 0	114 122	213 213	174 186	171 195
2011000341	104 122	111 129	188 196	80 80	104 106	76 96	102 102	125 125	86 94	0 0	116 128	213 217	174 182	171 193
2011000342	104 124	111 127	196 196	80 102	102 108	74 80	102 106	125 125	78 92	0 0	122 126	213 223	174 190	195 195
2011000343	94 104	113 113	188 196	80 80	104 104	72 94	104 104	105 125	78 78	0 0	114 116	213 213	190 198	0 0
2011000344	94 94	127 129	196 196	80 94	106 106	80 80	102 104	127 127	86 86	88 88	114 114	213 247	174 186	173 193
2011000346	108 124	113 127	192 196	80 80	98 102	74 80	102 106	125 125	86 94	0 0	126 132	213 243	174 194	173 193
2011000347	124 124	111 125	196 196	80 94	98 104	76 80	102 106	105 127	78 96	88 88	114 126	213 243	170 174	191 193
2011000348	104 108	113 127	196 196	100 100	104 112	80 94	102 102	127 127	86 86	0 0	114 116	213 213	178 194	191 191
2011000349	108 124	111 111	196 196	80 102	102 104	80 80	102 106	125 127	92 94	76 88	128 128	213 213	174 174	173 195
2011000350	124 124	111 127	188 194	80 80	112 112	76 76	106 106	125 127	86 88	0 0	114 126	229 245	174 174	171 191
2011000351	82 108	113 127	196 196	80 80	102 104	76 80	92 106	125 125	84 84	0 0	128 128	217 229	170 198	171 195
2011000352	104 108	111 111	196 196	80 94	102 108	76 76	100 102	127 127	86 86	84 88	124 128	213 213	170 198	173 195
2011000353	108 124	113 113	186 196	80 80	104 104	74 80	102 102	127 127	78 86	88 88	114 128	213 217	174 198	171 193
2011000354	104 124	113 113	192 196	80 80	102 112	74 80	104 106	127 131	86 86	80 88	122 128	213 213	174 178	171 191
2011000355	124 126	127 129	196 196	80 80	104 106	80 94	94 106	105 105	78 92	80 80	122 128	213 223	178 178	173 193
2011000356	94 122	111 113	196 196	80 80	104 110	76 94	104 106	127 127	86 94	80 88	122 126	213 229	174 182	193 193
2011000357	94 108	113 131	192 196	80 94	98 104	74 76	104 104	127 129	78 86	0 0	114 122	217 247	174 194	171 195
2011000358	104 104	113 131	188 196	80 80	104 104	74 76	102 104	105 129	78 78	0 0	126 128	213 229	174 190	187 193
2011000359	122 122	113 131	196 196	80 80	102 102	76 94	102 104	105 127	86 92	76 76	114 128	233 233	174 186	191 193
2011000360	94 124	113 131	196 196	80 80	102 102	74 76	92 104	105 127	78 92	0 0	114 128	233 233	190 194	0 0
2011000361	124 132	111 127	196 196	80 80	98 106	76 80	102 106	105 129	96 96	80 88	126 132	217 233	174 178	173 195
2011000362	122 124	111 127	196 196	80 80	104 106	80 80	102 102	125 127	78 86	76 88	122 128	213 223	174 178	193 195
2011000363	108 108	111 111	188 196	80 94	104 104	76 80	102 104	125 127	78 78	78 78	114 122	213 233	194 198	193 195
2011000364	108 124	111 127	196 196	94 94	104 104	80 94	102 102	127 127	86 86	76 88	122 132	213 213	174 174	191 195
2011000365	124 124	113 127	186 196	80 102	102 104	74 80	104 106	125 127	80 86	76 78	124 128	213 213	174 194	191 195
2011000366	94 108	111 127	196 196	80 80	98 102	74 76	102 104	125 135	86 92	88 88	114 128	213 217	178 190	191 193
2011000367	104 108	111 111	196 196	80 80	104 112	80 80	102 106	105 127	78 92	88 90	116 128	213 213	174 174	191 195
2011000368	108 122	113 113	194 194	80 80	102 104	76 80	102 102	127 127	78 78	80 88	122 128	213 215	174 174	171 193
2011000369	124 128	111 127	196 196	80 94	98 98	80 94	106 106	105 127	78 78	88 88	122 126	213 217	178 190	173 195
2011000370	104 122	113 125	196 196	80 80	102 104	80 80	102 108	125 129	86 86	76 88	114 114	227 243	198 198	171 193
2011000371	104 122	125 127	192 196	80 100	104 108	80 80	102 108	125 127	86 86	76 80	124 126	213 229	174 198	191 195
2011000372	124 124	111 113	196 196	80 80	98 102	76 80	104 106	127 127	80 86	76 82	124 128	213 217	198 198	195 195

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TAMUID	Leo006	Leo008	Leo031	Leo045	Leo077	Leo085	Leo098	Leo126	Leo224	Leo230	Leo247	Leo281	Leo391	Leo506
2011000373	104 124	111 129	194 196	80 80	102 106	80 94	96 102	125 127	86 92	0 0	122 132	213 223	174 174	0 0
2011000376	124 130	111 113	192 196	80 80	102 102	70 76	102 104	125 125	78 86	0 0	122 126	213 213	174 174	195 199
2011000377	94 104	111 113	194 196	80 94	98 108	76 80	102 104	125 127	78 86	88 88	116 128	213 243	174 190	191 191
2011000378	108 122	111 113	192 196	80 94	98 104	76 80	102 102	125 127	86 86	76 90	128 128	217 217	174 174	191 195
2011000379	104 104	111 127	196 196	80 80	102 112	74 80	102 106	127 131	86 96	0 0	122 122	213 251	174 194	195 195
2011000380	108 124	111 127	192 196	94 100	104 112	80 80	104 106	125 131	78 78	82 84	116 126	213 213	186 198	171 195
2011000383	94 104	129 131	196 196	80 80	98 108	76 80	102 102	105 125	86 86	84 90	114 114	213 213	174 198	171 193
2011000385	104 110	127 127	196 196	80 102	104 106	74 94	102 106	125 127	78 92	76 88	128 128	231 245	174 174	193 193
2011000387	104 104	127 127	196 196	80 80	102 104	80 80	104 108	131 143	78 86	78 82	114 116	213 217	174 190	191 193
2011000388	108 124	111 113	188 196	100 100	104 104	80 80	102 102	127 127	86 86	76 90	114 128	213 213	194 194	173 195
2011000389	108 124	127 131	192 196	102 102	104 106	94 94	102 106	105 105	86 86	76 76	122 122	213 213	174 198	193 193
2011000390	104 104	111 111	196 196	80 102	104 106	76 94	102 106	127 143	78 86	0 0	122 122	213 233	178 190	191 195
2011000391	94 108	113 131	196 196	80 80	104 110	76 94	102 104	125 129	78 86	82 88	114 116	213 213	174 174	191 193
2011000392	94 94	131 131	196 196	80 80	102 104	74 80	92 102	125 125	78 86	0 0	122 128	229 247	198 198	171 195
2011000393	100 124	113 127	196 196	80 80	98 102	80 80	92 108	127 127	78 94	80 88	116 128	213 213	166 198	193 195
2011000394	94 104	127 131	196 196	80 80	98 102	74 80	102 102	127 127	86 92	0 0	114 114	213 233	174 190	171 195
2011000395	94 94	113 127	192 196	80 80	104 104	74 80	102 104	105 127	86 86	0 0	122 122	213 213	174 190	171 193
2011000396	94 104	131 131	192 196	80 80	106 110	76 94	102 104	105 105	86 86	80 80	128 136	245 245	174 194	171 191
2011000397	96 122	127 131	196 196	80 80	102 104	74 80	102 104	125 125	86 86	76 82	128 132	217 247	198 198	171 171
2011000398	124 124	113 127	188 196	80 80	102 104	80 80	102 106	125 145	78 86	90 90	114 128	213 213	174 174	193 195
2011000399	108 128	111 111	188 196	80 94	98 106	80 80	102 106	105 127	86 86	76 90	116 124	217 245	174 174	195 195
2011000400	124 124	111 127	196 196	80 80	104 112	76 80	102 102	105 125	78 92	88 88	126 128	213 229	182 198	191 191
2011000401	94 124	127 131	188 196	80 80	98 104	76 80	102 104	105 127	78 86	76 88	122 126	213 247	174 186	191 193
2011000403	108 108	111 113	194 196	80 80	104 112	76 80	104 106	125 125	86 86	76 80	116 126	213 251	174 174	193 195
2011000405	94 94	113 127	196 196	80 80	102 104	80 94	102 104	105 125	92 94	0 0	114 116	223 229	170 174	191 195
2011000406	104 122	131 131	192 196	80 102	104 106	74 80	102 104	105 127	86 94	80 90	122 122	229 247	190 198	171 191
2011000408	108 124	111 111	188 196	80 80	102 104	76 80	92 106	127 145	78 86	76 80	114 116	213 223	174 190	191 191
2011000409	94 108	113 131	196 196	80 80	98 102	76 80	102 104	125 129	86 94	76 86	114 116	213 213	174 174	191 193
2011000410	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0
2011000411	104 124	111 133	188 194	80 80	102 106	80 92	94 108	125 125	86 86	80 88	114 132	223 223	174 174	171 191
2011000412	104 124	111 127	196 196	80 80	104 108	76 80	102 106	125 125	78 78	80 88	122 132	213 213	174 174	195 195
2011000413	94 104	111 127	192 196	80 80	102 102	80 94	92 104	129 129	86 94	0 0	116 128	217 247	174 198	191 197
2011000414	108 124	111 127	196 196	80 80	104 104	80 80	102 102	127 127	78 86	80 88	114 128	213 223	178 178	191 193
2011000415	108 124	111 127	188 196	80 102	98 102	80 80	102 106	127 129	86 94	80 90	114 128	213 223	174 178	191 193
2011000416	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0
2011000417	104 108	111 111	196 196	94 102	102 102	76 80	102 106	125 131	78 86	82 90	128 128	213 217	170 186	191 193
2011000418	104 108	111 111	196 196	94 102	102 102	76 80	102 106	125 131	78 86	80 88	128 128	213 217	170 186	191 193
2011000420	104 108	111 111	196 196	94 102	102 102	76 80	102 106	125 131	78 86	80 80	128 128	213 217	170 186	191 193

B.2.a: ZAMBIAN STR ALLELE CALLS (PAGE 5 OF 11)

TAMUID	Leo006	Leo008	Leo031	Leo045	Leo077	Leo085	Leo098	Leo126	Leo224	Leo230	Leo247	Leo281	Leo391	Leo506
2011000421	0 0	113 127	192 196	0 0	104 104	80 80	106 106	0 0	86 96	76 88	114 114	215 215	174 174	0 0
2011000422	108 124	111 113	196 196	80 102	104 104	80 80	106 106	127 131	78 86	76 88	128 128	213 217	182 190	171 191
2011000424	108 122	113 127	188 196	80 80	104 106	80 80	102 106	105 125	78 86	76 88	114 132	213 213	174 174	193 193
2011000425	108 132	111 127	196 196	80 80	104 104	80 80	102 104	127 127	94 96	88 88	128 132	213 217	178 190	173 193
2011000426	94 122	127 131	196 196	80 80	104 110	80 80	102 102	127 131	86 86	76 80	114 114	229 233	178 190	191 193
2011000427	104 108	111 111	196 196	94 102	102 102	76 80	102 106	125 131	78 86	80 88	128 128	213 217	170 186	191 193
2011000428	104 122	111 113	194 196	80 94	102 104	76 80	102 106	125 125	78 86	80 88	126 126	213 229	174 174	191 193
2011000429	94 122	113 131	188 196	80 80	98 104	80 94	104 106	105 105	78 94	76 90	128 128	217 247	194 198	171 193
2011000430	94 108	111 111	196 196	80 102	98 104	76 94	102 104	127 145	78 78	76 76	122 122	223 223	174 186	193 195
2011000431	108 124	111 125	196 196	80 80	104 104	80 80	102 104	127 127	94 96	76 90	128 132	213 245	174 178	193 195
2011000432	124 124	127 127	188 196	80 80	102 104	80 94	104 106	135 145	78 78	76 80	122 128	243 251	174 174	195 195
2011000433	104 124	113 127	196 196	80 96	102 102	80 80	92 102	125 131	86 96	76 88	124 128	213 213	174 174	191 193
2011000434	94 110	113 127	196 196	80 80	104 110	80 86	92 102	127 131	78 86	76 82	114 114	229 233	178 186	171 193
2011000435	128 136	111 127	192 196	80 108	104 104	80 80	102 102	127 135	86 86	82 90	126 126	213 217	174 194	193 193
2011000436	122 124	111 113	186 196	80 80	98 110	80 94	92 102	105 131	94 96	88 88	126 132	213 213	174 174	173 193
2011000437	104 124	111 127	188 196	80 94	104 112	76 76	104 106	127 127	78 88	78 90	114 128	229 243	174 174	171 173
2011000438	96 104	113 113	196 196	80 80	104 104	94 94	102 104	105 125	78 78	80 88	114 128	213 213	174 190	191 191
2011000440	94 108	127 131	0 0	80 94	104 106	80 96	102 102	127 129	78 92	0 0	126 128	211 213	174 186	0 0
2011000441	108 124	111 127	196 196	80 100	104 104	76 76	102 102	127 135	86 86	80 88	128 128	213 213	194 194	173 173
2011000442	108 124	111 127	196 196	80 94	104 104	80 80	102 104	105 127	86 92	76 80	114 114	213 213	194 194	193 195
2011000443	96 122	111 113	188 196	80 94	98 110	80 80	104 106	105 131	92 92	76 80	114 114	213 215	190 190	171 197
2011000444	104 108	111 113	192 196	80 94	104 112	74 80	104 104	131 143	78 86	76 88	122 128	213 213	178 198	191 191
2011000446	104 108	127 131	196 196	80 80	102 104	76 80	102 106	125 127	86 92	78 90	122 128	213 213	174 178	191 193
2011000447	94 104	113 113	196 196	80 80	102 104	74 80	92 108	125 127	86 92	76 80	116 126	229 245	174 174	171 191
2011000448	94 108	111 131	196 196	80 102	104 104	76 80	102 104	127 127	78 92	88 88	126 128	213 227	178 186	195 199
2011000449	104 108	111 127	196 196	80 94	102 104	76 80	106 108	125 125	78 92	78 82	114 126	213 223	174 190	195 195
2011000450	122 124	131 131	192 192	80 102	104 106	80 94	102 106	105 127	86 94	88 88	122 128	213 227	174 198	191 193
2011000452	94 96	111 131	196 196	80 80	104 104	80 80	102 106	105 125	86 86	88 88	116 128	227 245	174 198	171 193
2011000453	108 108	113 113	194 196	80 80	102 112	76 80	102 104	125 125	78 92	80 88	116 122	213 229	174 198	191 193
2011000454	122 122	127 131	196 196	80 80	104 104	80 80	102 106	105 125	78 94	88 88	114 116	229 245	174 198	191 193
2011000455	108 108	111 111	196 196	80 100	102 104	76 80	106 106	125 125	78 86	80 84	116 126	213 213	174 186	173 195
2011000456	108 122	113 127	196 196	80 100	102 104	80 80	102 102	127 135	86 86	76 80	126 126	213 217	174 194	193 195
2011000457	94 110	111 113	196 196	80 80	102 104	76 80	102 104	105 127	78 94	78 92	122 128	213 213	174 186	191 195
2011000458	108 122	113 113	196 196	80 94	104 112	74 80	102 106	125 127	78 92	76 88	116 128	231 245	174 174	173 199
2011000459	108 108	111 111	196 196	80 80	104 104	80 80	102 104	127 127	78 78	76 88	114 128	213 243	174 190	193 199
2011000460	124 124	113 127	188 196	80 80	102 104	76 80	104 108	125 127	78 78	90 90	126 128	213 213	174 198	191 193
2011000461	94 122	111 131	196 196	80 80	106 110	74 76	102 104	125 125	78 86	82 90	116 128	213 213	182 186	171 193
2011000462	96 108	127 131	196 196	80 102	102 104	76 94	104 104	125 129	78 92	90 90	114 128	213 247	174 194	173 193

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TAMUID	Leo006	Leo008	Leo031	Leo045	Leo077	Leo085	Leo098	Leo126	Leo224	Leo230	Leo247	Leo281	Leo391	Leo506
2011000463	94 110	113 127	196 196	80 80	98 106	74 80	102 104	105 105	78 78	76 88	126 128	213 213	174 198	191 193
2011000465	108 124	127 127	196 196	94 94	102 112	80 80	102 104	127 129	78 78	90 90	114 116	213 229	170 198	195 195
2011000466	94 94	113 127	196 196	80 80	102 104	80 80	104 104	129 143	86 86	88 90	114 116	213 229	174 178	191 195
2011000467	82 108	111 113	196 196	80 96	102 104	76 94	102 104	125 127	78 86	88 90	124 126	213 229	174 174	191 199
2011000468	108 124	113 127	192 196	80 100	102 104	76 80	102 106	127 135	86 86	76 80	126 126	213 217	174 194	193 195
2011000470	104 130	111 127	192 196	80 80	102 102	80 94	92 104	105 125	86 94	82 88	116 128	217 245	198 198	191 193
2011000471	108 124	113 113	188 194	80 100	102 102	80 94	102 106	125 125	86 92	76 88	128 128	213 213	174 174	193 199
2011000472	108 108	111 113	196 196	80 80	98 102	80 80	102 106	125 127	78 78	80 88	124 128	213 223	170 174	171 195
2011000473	122 122	113 113	196 196	80 94	104 112	80 80	102 102	125 125	78 86	88 88	126 128	213 217	174 198	171 171
2011000474	104 122	113 131	196 196	80 94	98 106	80 80	92 102	127 127	78 86	0 0	128 128	213 213	174 198	171 191
2011000475	122 122	111 111	196 196	80 80	104 104	76 94	102 106	105 125	78 86	0 0	128 128	217 229	174 174	195 195
2011000476	94 122	113 131	192 192	80 80	102 106	74 80	104 106	125 129	86 94	82 90	128 128	213 245	174 178	171 191
2011000477	104 104	111 131	188 196	80 80	104 104	80 80	102 104	105 129	92 94	78 88	116 122	231 245	182 194	171 197
2011000478	124 124	113 127	196 196	80 80	104 112	80 80	104 104	125 131	86 86	88 88	114 128	213 229	174 198	171 193
2011000480	122 124	113 127	196 196	80 80	102 104	76 80	102 104	125 125	78 96	80 90	128 132	213 213	174 198	173 193
2011000481	104 108	131 131	196 196	80 80	104 104	94 94	104 104	105 127	86 94	82 88	122 128	211 247	174 198	171 193
2011000482	104 122	113 127	196 196	80 80	104 104	74 94	104 104	105 127	78 92	76 88	128 128	213 233	190 194	191 195
2011000483	104 122	111 111	196 196	80 100	98 104	94 94	102 102	125 125	86 96	80 88	128 128	213 213	174 178	173 195
2011000484	94 104	127 131	196 196	80 80	102 104	80 86	102 104	125 127	78 78	80 90	114 116	213 217	174 190	171 191
2011000485	108 124	111 111	196 196	80 100	102 104	80 80	102 106	125 127	78 86	80 90	122 128	213 223	170 178	193 193
2011000486	96 104	113 131	196 196	80 80	104 104	76 94	104 106	127 127	78 94	82 90	122 126	227 245	174 190	193 197
2011000487	104 122	127 131	196 196	80 80	102 104	76 94	102 102	125 125	92 94	76 76	114 128	213 233	182 198	171 197
2011000488	96 122	125 127	196 196	80 80	102 102	80 86	94 108	105 127	86 94	0 0	126 128	213 245	186 190	0 0
2011000489	122 130	113 127	196 196	80 80	102 104	80 80	104 106	127 127	86 92	76 90	124 128	213 215	182 186	193 193
2011000491	108 124	111 125	196 196	80 94	98 106	80 94	102 106	125 127	86 92	76 88	124 132	217 233	178 178	191 193
2011000492	124 132	111 113	196 196	80 80	106 106	76 80	102 106	103 125	86 96	88 92	126 132	217 231	178 194	191 195
2011000493	94 122	127 127	192 196	80 80	102 104	80 94	104 104	129 129	92 94	76 90	114 128	213 217	186 194	193 193
2011000494	104 104	111 131	196 196	80 80	104 106	80 94	102 106	105 125	86 86	90 90	122 122	245 245	186 190	0 0
2011000495	122 124	113 113	196 196	80 100	108 112	76 80	102 102	125 131	78 78	76 88	114 122	213 213	174 198	191 191
2011000496	94 110	131 131	196 196	80 80	98 106	76 80	104 108	105 125	86 94	74 90	122 128	213 213	182 186	193 193
2011000497	82 122	111 111	196 196	80 80	98 112	76 80	106 106	127 129	78 86	76 88	116 126	213 233	174 174	193 195
2011000498	82 108	125 127	196 196	80 80	104 104	76 94	102 102	105 127	86 86	76 88	114 132	213 213	174 178	191 193
2011000499	124 124	113 127	196 196	80 80	102 104	80 80	102 104	105 143	78 86	90 94	114 128	213 245	174 174	195 195
2011000500	122 122	127 127	192 196	80 80	98 104	76 76	102 106	105 125	86 94	76 76	114 128	213 213	174 174	193 193
2011000501	94 104	131 131	196 196	80 94	104 104	74 80	102 104	105 129	86 94	74 90	128 128	211 245	178 182	171 193
2011000502	104 124	127 127	196 196	80 80	102 102	80 94	102 102	125 131	78 78	80 88	114 122	213 223	174 190	193 193
2011000503	108 124	111 127	196 196	94 100	104 104	80 80	102 104	105 127	86 92	76 80	114 128	213 213	194 194	173 195
2011000684	104 126	113 127	192 196	80 104	104 110	80 80	104 106	105 125	78 86	76 88	114 128	211 213	174 186	193 195

B.2.a: ZAMBIAN STR ALLELE CALLS (PAGE 7 OF 11)

TAMUID	Leo006	Leo008	Leo031	Leo045	Leo077	Leo085	Leo098	Leo126	Leo224	Leo230	Leo247	Leo281	Leo391	Leo506
2011000685	94 122	113 127	192 196	80 80	106 110	80 80	106 106	125 129	86 86	78 90	126 128	213 247	174 174	171 195
2011000686	104 108	111 131	196 196	80 94	102 108	76 76	102 106	127 127	86 86	76 80	122 128	213 213	170 174	173 193
2011000687	104 108	127 131	196 196	80 80	102 104	76 80	102 106	125 125	86 92	76 88	122 128	213 213	174 178	173 193
2011000688	104 108	111 113	196 196	80 80	104 112	74 80	104 104	131 145	78 86	88 88	122 128	213 213	178 198	171 191
2011000690	108 124	113 129	196 196	100 102	102 104	80 80	106 106	125 131	86 86	0 0	128 128	213 213	174 182	171 195
2011000691	108 108	113 113	196 196	80 80	102 112	94 94	106 106	125 127	78 96	76 76	126 128	213 213	174 174	171 191
2011000692	104 124	111 127	196 196	80 94	102 112	76 76	102 102	125 125	86 86	80 88	128 128	217 243	186 190	193 193
2011000693	122 124	111 111	194 196	80 94	104 104	76 80	104 106	125 127	78 78	78 84	124 126	213 229	186 198	171 173
2011000694	108 122	111 113	196 196	94 94	104 104	80 80	102 106	135 145	86 92	82 90	116 128	213 213	174 178	173 191
2011000695	124 124	111 111	188 196	80 100	102 104	76 80	102 102	127 135	86 92	80 90	114 128	213 229	178 194	173 195
2011000696	82 108	111 125	188 196	80 94	98 106	80 94	102 106	127 127	86 92	76 88	124 128	217 245	174 178	191 195
2011000698	108 128	111 129	196 196	80 80	98 104	80 80	102 102	103 125	86 96	0 0	116 128	213 217	174 190	191 195
2011000699	108 122	111 113	196 196	94 94	104 104	80 80	102 106	135 143	86 92	84 90	116 128	213 213	174 178	173 191
2011000703	104 108	111 111	188 196	94 100	106 106	76 80	106 106	105 125	78 86	76 88	114 126	213 245	174 178	193 195
2011000704	94 108	111 113	188 196	94 102	98 104	76 80	102 104	125 127	78 78	88 88	114 116	233 245	174 190	191 195
2011000705	104 108	113 127	196 196	80 100	104 104	76 80	102 102	105 127	86 86	76 88	114 128	213 213	178 194	173 193
2011000706	94 104	111 113	190 196	80 96	104 104	80 80	102 106	125 131	78 78	80 88	116 128	213 223	170 194	191 191
2011000707	104 108	111 111	196 196	80 100	104 108	76 80	102 106	129 143	78 94	76 88	122 132	213 247	174 198	173 191
2011000708	82 130	127 127	196 196	80 80	104 112	76 80	102 106	105 129	86 92	80 90	114 128	213 213	174 190	173 191
2011000709	108 108	111 113	196 196	80 80	104 104	74 76	102 102	125 131	78 78	76 88	126 128	229 249	182 198	191 195
2011000710	108 108	113 127	196 196	80 80	104 102	74 74	102 102	105 105	78 78	76 88	126 128	213 213	186 198	173 191
2011000711	104 122	111 127	196 196	80 94	102 108	74 80	102 106	125 131	78 86	78 90	114 126	213 229	174 190	171 195
2011000712	104 124	113 113	196 196	80 94	102 104	80 94	102 104	127 127	78 96	88 88	128 128	213 229	174 198	195 195
2011000713	104 108	113 113	194 196	80 94	102 112	74 80	102 106	127 127	86 86	76 88	126 128	213 213	174 174	171 171
2011000714	104 122	113 113	196 196	80 94	104 104	74 76	102 106	127 127	78 86	82 90	126 128	213 213	170 174	173 195
2011000715	122 124	113 127	186 188	80 94	104 112	76 80	104 106	125 127	86 88	76 88	114 126	213 243	174 174	191 195
2011000716	124 124	113 127	196 196	80 80	104 112	80 80	104 104	125 131	86 86	88 88	114 128	213 229	174 198	171 193
2011000717	108 122	113 113	196 196	80 94	104 112	74 80	102 106	125 127	86 86	76 88	126 128	213 213	170 174	191 195
2011000718	104 124	111 113	194 196	80 100	102 106	74 76	102 104	125 127	78 78	90 90	122 128	213 229	174 190	171 195
2011000719	108 124	111 125	196 196	80 80	104 108	76 80	102 104	103 123	78 78	88 88	114 128	213 217	174 190	195 195
2011000720	124 124	111 113	186 194	80 94	102 112	76 80	106 106	125 127	86 88	76 88	114 126	213 229	174 174	191 193
2011000723	108 124	127 127	196 196	80 94	104 112	76 80	102 104	127 131	86 92	82 88	126 128	213 213	198 198	171 191
2011000724	108 124	111 127	196 196	80 80	104 104	74 76	102 102	105 131	78 78	88 88	114 128	213 229	198 198	171 191
2011000725	124 124	111 113	192 196	80 94	104 104	74 76	102 104	125 131	78 86	82 88	114 126	213 229	186 198	173 195
2011000727	124 124	111 111	192 196	0 0	106 112	76 80	106 108	127 127	78 86	76 80	114 128	213 213	170 170	0 0
2011000729	104 104	113 113	196 196	80 80	104 104	80 94	104 108	125 129	78 92	76 90	116 116	213 213	182 198	171 191
2011000730	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0
2011000732	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0

B.2.a: ZAMBIAN STR ALLELE CALLS (PAGE 8 OF 11)

TAMUID	Leo006	Leo008	Leo031	Leo045	Leo077	Leo085	Leo098	Leo126	Leo224	Leo230	Leo247	Leo281	Leo391	Leo506
2011000733	94 94	113 131	192 196	80 80	102 104	74 80	92 104	125 127	86 94	82 88	126 128	213 247	174 198	171 191
2011000735	124 132	111 127	196 196	80 80	98 106	76 80	102 106	105 129	96 96	82 90	126 132	217 231	174 178	173 195
2011000736	104 104	131 131	196 196	80 102	104 106	80 94	102 102	125 127	86 94	76 88	114 122	213 247	174 186	193 193
2011000737	100 104	127 127	188 196	80 94	102 104	76 94	102 102	125 131	86 94	78 82	128 132	217 223	186 198	191 197
2011000738	94 110	111 131	188 196	80 80	104 106	80 80	104 106	125 127	78 86	76 80	128 128	245 245	174 174	191 197
2011000739	94 96	111 113	188 196	80 80	106 106	74 80	104 106	125 127	78 86	76 80	126 128	247 247	174 174	193 197
2011000740	94 96	111 131	188 196	80 80	106 106	74 80	92 104	125 127	78 86	76 80	126 128	217 233	174 174	171 191
2011000741	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0
2011000742	96 110	111 131	196 196	80 80	104 106	74 80	94 106	127 127	78 86	80 88	128 128	217 245	174 198	191 193
2011000743	104 104	113 113	196 196	80 80	104 104	80 94	104 108	125 129	78 92	76 90	116 116	213 213	182 198	171 191
2011000744	104 104	113 131	192 196	80 80	104 104	80 94	102 108	127 129	86 92	76 76	116 128	213 229	174 182	171 171
2011000745	96 96	131 131	196 196	80 80	104 104	74 80	92 108	127 127	78 86	76 80	114 128	213 217	174 182	187 191
2011000746	104 122	111 131	188 196	80 100	102 104	80 80	106 106	125 127	78 92	76 82	128 128	213 247	178 198	171 193
2011000748	104 110	127 131	196 196	80 80	104 104	86 94	94 102	127 127	86 94	80 80	126 128	229 233	178 190	171 195
2011000749	122 122	113 113	188 196	80 80	98 102	74 94	104 104	127 129	86 86	76 82	114 116	213 213	190 190	171 171
2011000750	122 124	113 131	188 196	80 80	98 102	74 76	102 104	125 129	86 86	80 88	114 116	213 213	174 198	171 171
2011000751	122 124	111 131	192 196	80 80	98 104	80 80	102 104	105 125	78 94	82 88	114 114	213 213	190 198	171 193
2011000752	122 122	113 131	188 196	80 80	98 102	74 76	102 104	125 129	86 86	90 90	114 116	213 213	174 198	171 171
2011000753	100 122	127 127	196 196	80 80	102 104	80 80	102 102	105 125	78 94	78 90	114 122	217 229	174 182	193 197
2011000754	94 94	113 127	192 196	80 80	104 106	74 80	102 102	105 127	78 86	76 90	114 128	211 229	190 198	171 195
2011000757	104 122	127 127	192 196	80 94	104 104	80 96	102 104	127 127	78 86	78 90	116 128	213 233	174 190	191 195
2011000759	96 96	113 131	188 196	80 80	104 104	76 76	102 104	125 129	86 86	76 88	122 128	215 233	174 174	171 193
2011000761	82 122	131 131	192 196	80 80	104 106	80 94	102 104	105 129	86 94	76 76	128 128	233 245	174 190	171 197
2011000762	94 96	111 113	188 196	80 80	106 106	74 80	104 106	125 127	78 86	76 80	126 128	245 245	174 174	193 197
2011000763	96 108	113 131	188 196	80 80	98 110	80 86	104 104	125 127	86 94	80 80	116 128	245 245	186 190	171 173
2011000764	108 124	113 127	196 196	80 80	102 110	76 76	102 104	105 105	78 92	76 76	122 122	213 229	190 198	191 197
2011000765	96 122	127 131	196 196	80 80	102 104	74 80	102 104	125 125	86 86	76 82	128 132	217 245	198 198	171 171
2011000766	0 0	0 0	0 0	0 0	0 0	76 78	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0
2011000767	108 130	131 131	192 196	80 94	98 104	74 76	92 108	127 129	78 86	76 88	128 132	213 229	186 194	171 191
2011000768	104 104	127 131	196 196	80 94	98 110	76 80	102 104	105 127	78 86	0 0	116 128	213 247	198 198	193 193
2011000769	104 108	127 131	196 196	80 80	104 110	76 94	104 104	105 105	78 86	78 88	116 122	245 245	190 198	171 193
2011000770	124 124	113 127	196 196	80 80	104 112	80 80	104 104	125 131	86 86	88 88	114 128	213 229	174 198	171 193
2011000772	96 122	113 127	196 196	80 80	102 104	74 80	104 108	105 129	86 86	74 76	114 114	213 213	174 190	171 171
2011000773	94 122	113 127	192 196	80 102	98 104	80 94	102 104	129 131	86 86	82 88	114 128	213 213	0 0	193 195
2011000775	94 104	111 113	188 196	80 80	104 106	76 80	104 104	125 129	78 92	76 88	122 122	229 247	174 174	173 187
2011000776	94 104	127 131	196 196	80 80	102 104	80 86	102 104	125 127	78 78	80 90	114 116	213 217	174 190	171 191
2011000777	96 104	111 113	196 196	80 80	98 102	80 80	102 104	125 127	78 86	80 88	122 128	215 233	178 186	173 197
2011000778	0 0	0 0	0 0	0 0	0 0	78 78	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0

B.2.a: ZAMBIAN STR ALLELE CALLS (PAGE 9 OF 11)

TAMUID	Leo006	Leo008	Leo031	Leo045	Leo077	Leo085	Leo098	Leo126	Leo224	Leo230	Leo247	Leo281	Leo391	Leo506
2011000779	94 122	131 131	192 196	80 80	104 104	94 94	102 106	105 127	86 86	76 88	122 122	213 229	174 194	191 193
2011000780	104 108	113 127	196 196	80 80	104 110	80 80	104 106	127 129	78 86	82 90	114 128	213 245	182 190	193 195
2011000781	104 122	113 131	192 196	80 100	104 106	76 80	102 102	105 129	94 94	78 90	122 128	213 245	174 186	173 193
2011000782	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0
2011000783	94 122	113 127	192 196	80 80	102 104	74 80	108 108	105 105	78 94	80 88	114 128	213 215	186 198	193 195
2011000784	96 122	113 127	196 196	80 80	98 104	74 80	104 108	127 127	86 86	74 82	114 116	213 213	174 190	171 195
2011000785	94 104	113 113	196 196	80 80	104 104	80 80	102 104	125 127	86 92	80 88	128 128	213 213	174 174	171 193
2011000786	94 104	113 113	196 196	80 80	104 106	80 94	102 104	105 127	78 86	82 88	128 128	213 213	174 174	171 191
2011000787	94 110	127 131	196 196	80 80	102 104	80 80	94 102	125 127	86 86	74 80	126 128	213 213	174 190	171 195
2011000788	108 122	111 131	0 0	80 80	104 106	80 94	92 102	125 125	78 86	76 90	122 122	213 249	174 186	193 193
2011000789	104 122	111 127	192 196	80 80	104 104	80 94	102 102	105 125	86 94	88 88	122 122	229 247	174 190	171 193
2011000790	104 122	113 131	188 196	80 80	104 104	74 74	102 104	105 129	78 78	76 88	128 128	213 213	174 194	193 193
2011000791	94 104	129 131	196 196	80 80	98 108	80 80	102 102	105 125	86 86	84 90	114 116	213 213	174 198	171 193
2011000793	94 94	127 131	196 196	94 102	104 104	76 80	104 106	127 129	78 78	82 88	122 122	213 213	186 186	191 195
2011000794	94 122	127 131	192 196	102 104	104 106	80 80	102 104	105 127	86 94	84 90	122 128	213 233	190 198	191 195
2011000795	94 104	113 127	196 196	80 80	98 104	76 80	102 106	125 125	80 86	82 90	114 116	213 217	182 190	171 193
2011000796	94 94	127 127	192 196	80 80	98 106	80 80	102 108	127 131	78 94	88 88	114 114	229 247	186 198	171 195
2011000797	96 122	113 127	196 196	80 80	98 104	80 80	102 102	105 105	86 94	76 90	114 122	229 245	174 186	195 197
2011000798	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	72 90	0 0	0 0	0 0	0 0
2011000799	94 94	113 127	192 196	80 80	98 106	74 80	102 104	125 127	78 94	76 90	114 128	229 245	186 198	171 195
2011000800	94 94	127 127	192 196	80 80	98 106	80 80	102 108	127 131	78 94	88 88	114 114	229 247	186 198	171 195
2011000801	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0
2011000802	104 110	111 127	196 196	80 80	102 104	80 80	92 108	125 127	86 86	74 80	126 132	213 229	186 190	195 195
2011000803	96 104	127 131	192 196	80 80	106 108	74 76	104 106	125 127	78 78	76 88	122 126	217 229	174 174	171 197
2011000804	96 104	131 131	192 196	80 102	106 110	74 76	102 104	125 127	90 92	78 90	122 126	217 233	174 174	171 197
2011000805	104 104	127 131	196 196	80 80	104 104	80 94	102 102	125 129	92 94	78 88	114 128	213 229	174 182	171 197
2011000806	104 104	127 131	188 196	80 80	102 104	76 80	102 102	105 105	78 86	0 0	122 132	213 229	190 198	171 193
2011000807	104 104	127 131	196 196	80 94	98 110	76 80	102 104	105 127	78 86	80 80	116 128	213 245	198 198	193 193
2011000809	104 124	131 131	196 196	80 80	104 104	80 94	104 106	105 127	86 86	76 82	116 122	213 245	190 198	191 193
2011000810	96 104	111 113	188 188	80 80	104 106	76 80	102 104	125 129	86 92	78 90	122 128	213 229	174 186	187 193
2011000811	94 104	127 131	196 196	80 80	104 106	80 80	102 104	127 127	92 92	80 88	114 116	213 213	174 186	173 193
2011000812	94 130	127 131	196 196	80 80	104 106	74 80	104 108	127 129	78 92	80 88	114 124	213 213	174 186	173 193
2011000813	122 122	113 127	196 196	80 80	102 104	80 80	102 104	127 127	86 86	80 88	114 114	213 229	190 198	173 193
2011000814	104 104	131 131	192 196	80 80	104 108	76 80	102 106	125 127	78 92	76 88	128 128	213 217	190 198	171 193
2011000815	122 122	129 131	192 196	80 80	104 108	80 80	102 104	105 105	78 86	80 88	114 114	213 229	178 198	171 191
2011000816	104 104	111 113	192 196	80 80	102 104	80 80	104 104	105 127	78 86	76 90	114 116	215 245	174 174	173 193
2011000817	104 110	127 131	188 196	80 102	104 104	74 74	102 106	125 127	78 86	76 82	128 128	213 233	174 178	193 197
2011000818	94 104	111 129	192 196	80 80	98 104	80 80	102 102	105 127	78 86	84 90	114 116	213 213	174 198	171 191

B.2.a: ZAMBIAN STR ALLELE CALLS (PAGE 10 OF 11)

TAMUID	Leo006	Leo008	Leo031	Leo045	Leo077	Leo085	Leo098	Leo126	Leo224	Leo230	Leo247	Leo281	Leo391	Leo506
2011000819	122 122	111 131	196 196	94 94	104 104	76 80	106 108	105 125	86 92	74 80	124 126	211 215	174 190	171 195
2011000820	122 122	111 131	196 196	80 94	98 104	80 80	102 106	105 125	92 94	76 90	116 126	211 229	186 190	171 195
2011000821	94 108	111 113	196 196	80 94	98 102	76 76	102 106	125 127	78 86	0 0	122 126	213 217	174 186	173 195
2011000822	94 104	111 127	188 196	80 94	102 112	76 80	104 104	125 143	78 78	80 88	114 126	213 213	174 182	191 193
2011000823	108 124	111 127	188 196	80 80	106 112	76 80	106 106	125 125	86 86	76 88	126 128	217 229	174 174	173 191
2011000824	104 108	113 127	188 196	80 94	104 104	76 80	104 106	125 127	88 96	88 88	126 128	217 243	174 174	173 193
2011000825	124 124	111 127	188 196	80 102	102 112	76 76	106 106	125 127	78 88	80 88	114 114	213 229	174 174	171 189
2011000826	108 124	111 111	196 196	80 94	104 112	76 80	106 106	125 127	88 96	76 88	114 116	229 247	174 174	173 193
2011000827	108 124	111 113	196 196	94 94	102 112	76 80	102 104	127 129	78 78	82 88	114 116	213 213	186 198	195 195
2011000828	108 124	111 127	196 196	80 80	98 104	80 80	102 102	105 125	86 92	76 88	128 132	213 213	178 178	173 195
2011000829	108 124	111 113	196 196	80 96	104 106	76 80	102 104	125 129	86 94	88 90	124 128	213 213	174 174	171 193
2011000830	104 124	113 131	196 196	80 80	102 108	80 80	104 108	131 143	78 86	76 88	114 116	217 223	174 190	193 193
2011000831	108 124	113 129	196 196	80 80	104 106	80 94	100 104	105 125	78 92	80 90	128 132	213 223	174 174	171 191
2011000832	108 124	113 129	196 196	80 80	104 104	80 80	102 104	105 143	78 86	80 90	114 132	213 223	174 174	171 195
2011000833	94 122	111 127	196 196	80 80	104 104	76 80	104 108	105 125	92 92	74 80	116 122	211 245	174 174	191 195
2011000834	108 124	111 129	194 196	94 94	98 106	80 94	100 106	105 105	86 92	90 94	126 128	223 251	174 174	171 191
2011000835	94 124	111 127	188 196	80 80	108 112	76 76	104 106	125 127	86 86	76 84	114 114	213 229	174 174	173 191
2011000836	108 124	113 127	196 196	96 100	102 106	80 80	102 104	125 127	78 94	76 88	124 128	213 217	170 174	171 195
2011000837	108 124	111 113	196 196	80 80	106 108	76 80	102 104	125 125	78 78	88 88	116 128	213 213	174 190	191 195
2011000838	108 124	113 125	196 196	80 80	102 108	76 76	102 102	125 125	78 86	76 76	122 128	213 243	174 174	191 195
2011000839	108 108	111 111	196 196	80 94	104 104	80 94	102 102	105 105	86 92	88 90	116 132	213 243	174 198	191 193
2011000840	108 108	111 113	194 196	80 80	98 112	76 94	102 104	125 125	78 92	76 88	122 122	217 229	174 198	191 193
2011000841	104 108	111 127	196 196	100 102	98 104	80 94	102 102	127 127	86 86	76 90	114 116	213 217	190 194	173 191
2011000842	94 108	113 113	196 196	80 94	102 106	80 80	102 102	127 143	86 96	88 90	126 128	213 247	178 190	191 191
2011000844	104 122	113 131	192 196	80 80	104 106	80 80	104 106	105 129	78 86	76 88	114 128	213 213	174 198	171 193
2011000845	104 104	113 113	188 192	80 80	104 106	80 80	92 102	105 125	78 78	76 88	114 122	213 213	190 198	191 193
2011000846	94 122	127 131	196 196	80 80	102 106	80 94	102 104	127 129	78 86	74 90	116 128	213 213	186 198	193 195
2011000847	104 124	127 127	192 196	80 80	102 104	74 80	102 104	105 127	78 78	88 88	114 116	213 223	186 186	173 181
2011000848	104 124	111 127	196 196	80 94	102 104	94 94	102 102	125 127	86 86	80 84	114 128	223 247	174 198	195 199
2011000849	108 124	111 127	188 196	80 100	102 102	80 94	102 104	125 127	86 86	88 90	124 128	213 253	174 194	181 195
2011000850	104 124	111 127	196 196	80 80	102 104	80 94	102 106	125 127	94 96	76 88	126 128	213 213	174 186	191 193
2011000851	108 124	111 113	188 194	80 94	102 104	76 80	106 106	105 127	78 86	80 88	116 126	213 229	174 174	171 193
2011000852	94 108	111 111	196 196	94 94	102 102	76 76	106 106	127 127	78 86	76 76	126 126	213 213	174 186	173 173
2011000854	94 108	127 131	192 196	80 80	106 110	76 80	102 104	125 131	90 92	76 88	116 126	213 213	174 182	171 195
2011000855	108 122	111 131	196 196	80 80	104 106	80 94	92 102	125 125	86 94	76 90	122 122	213 247	174 186	193 193
2011000856	104 108	111 113	196 196	100 100	102 104	76 80	102 106	105 127	94 96	76 80	122 128	213 213	174 194	173 193
2011000857	122 124	111 111	196 196	80 100	102 104	76 94	102 102	125 125	86 92	76 88	122 126	213 217	174 178	173 195
2011000858	82 108	111 113	188 196	80 80	104 106	76 80	102 106	127 127	86 92	76 76	124 132	233 233	174 178	191 193

B.2.a: ZAMBIAN STR ALLELE CALLS (PAGE 11 OF 11)

TAMUID	Leo006	Leo008	Leo031	Leo045	Leo077	Leo085	Leo098	Leo126	Leo224	Leo230	Leo247	Leo281	Leo391	Leo506
2011000859	108 108	111 111	188 196	80 80	98 104	76 94	104 106	125 127	78 86	76 76	122 132	217 233	174 198	191 195
2011000860	108 122	111 113	188 196	80 80	102 104	76 80	102 102	127 127	86 92	88 90	128 132	213 229	174 178	195 195
2011000861	122 124	111 113	192 196	100 100	102 104	80 94	102 104	125 127	86 94	88 88	114 114	213 243	174 178	191 191
2011000862	124 124	111 127	188 196	80 94	104 104	76 80	102 102	105 127	92 94	76 76	114 124	217 243	174 174	191 193
2011000863	104 124	111 111	196 196	80 80	98 102	80 94	102 102	125 129	86 86	80 82	114 128	213 223	174 194	191 195
2011000864	94 128	113 113	196 196	80 80	102 104	76 80	102 102	105 105	78 86	90 90	114 122	213 213	174 190	195 199
2011000865	104 124	113 125	196 196	80 94	104 110	76 94	102 102	105 127	92 96	78 90	128 128	213 215	178 190	193 193
2011000866	94 108	111 113	194 196	80 94	104 112	80 80	102 106	105 127	78 92	78 82	122 128	213 215	174 174	193 193
2011000867	104 108	111 111	196 196	80 80	98 98	76 80	102 106	127 127	78 86	78 90	114 126	213 233	174 174	171 195
2011000868	122 124	111 127	194 196	80 80	102 104	80 80	102 104	127 143	78 92	92 92	114 124	217 223	174 174	173 191
2011000869	104 122	113 131	188 192	80 94	104 104	74 94	104 104	105 129	86 92	78 90	128 128	233 245	190 194	191 193
2011000870	94 122	127 131	188 196	80 80	102 104	74 94	104 104	105 127	86 92	78 78	128 128	233 245	174 190	191 193
2011000871	124 128	111 127	196 196	80 80	102 104	80 80	102 106	105 127	92 96	92 92	114 126	213 233	174 190	193 195
2011000872	122 124	113 127	188 196	80 80	104 112	74 80	102 102	125 125	92 96	78 90	114 128	217 243	170 174	193 193
2011000873	108 108	111 125	196 196	80 80	102 104	80 80	102 102	105 105	78 86	78 82	122 126	213 229	174 174	191 195
2011000874	108 124	113 125	196 196	80 80	104 110	76 94	102 104	105 105	86 92	78 82	128 130	213 215	174 178	193 195
2011000875	108 124	111 113	188 196	80 80	98 104	76 80	102 102	125 127	86 92	78 84	126 126	217 233	174 178	191 193
2011000876	82 104	111 111	196 196	80 96	104 104	80 94	102 102	125 125	86 92	92 92	114 116	213 217	174 178	191 191
2011000877	108 124	111 111	196 196	80 80	98 104	76 80	102 102	125 127	86 86	84 92	126 132	213 213	174 178	173 191
2011000878	94 94	111 127	188 196	80 102	98 104	74 94	104 104	127 129	78 78	78 96	114 114	213 213	174 190	171 171
2011000879	108 122	113 131	192 196	80 96	102 104	76 80	106 106	125 131	78 86	90 90	116 128	233 243	174 186	173 195
2011000880	104 124	111 127	196 196	80 102	102 104	76 80	94 104	105 105	88 96	78 92	122 126	213 213	174 174	171 173
2011000881	122 124	111 127	196 196	80 96	98 110	80 80	102 104	127 143	96 96	90 92	126 128	213 213	174 198	193 195
2011000882	94 104	111 127	196 196	80 102	102 102	94 94	104 108	129 129	78 92	84 90	116 128	245 245	186 190	195 195
2011000883	94 94	127 131	188 196	80 80	104 108	74 94	102 104	129 129	78 94	84 90	116 122	213 213	186 186	193 195
2011000884	104 124	111 129	196 196	80 80	104 106	76 80	102 104	127 131	78 86	82 92	122 128	213 213	174 174	193 193
2011000885	104 130	111 113	192 196	72 80	104 106	76 80	94 106	125 127	78 78	90 90	128 128	213 213	174 178	171 191
2011000886	108 124	111 127	192 196	80 100	104 104	80 80	102 102	127 135	86 86	82 84	122 126	213 213	174 194	193 193
2011000887	108 124	111 111	188 196	94 94	106 106	80 94	106 106	105 127	86 92	78 90	122 124	213 217	178 178	193 195

B.2.b: MODERN POPULATION STR ALLELE CALLS (4 PAGES)

ID	Leo006	Leo008	Leo085	Leo098	Leo126	Leo224	Leo230	Leo247	Leo281	FCA026	FCA032	FCA075	FCA091	FCA094	FCA208	FCA275
M_GIR_001	94 94	113 113	80 80	106 106	129 131	92 92	78 78	130 130	223 223	150 150	196 196	131 131	154 154	222 222	326 326	153 153
M_GIR_002	94 94	113 113	80 80	106 106	129 129	98 98	78 78	130 130	223 223	150 150	196 196	131 131	154 154	222 222	326 326	153 153
M_GIR_003	94 94	113 113	80 80	106 106	129 131	92 92	78 78	130 130	223 223	150 150	196 196	131 131	154 154	222 222	326 326	153 153
M_GIR_004	94 94	113 113	80 80	106 106	129 131	92 92	78 78	130 130	223 223	150 150	196 196	131 131	154 154	222 222	326 326	153 153
M_GIR_005	94 94	113 113	80 80	106 106	129 131	92 92	78 78	130 130	223 223	150 150	196 196	131 131	154 154	222 222	326 326	153 153
M_GIR_006	94 94	113 113	80 80	106 106	129 129	92 98	78 78	130 130	223 223	150 150	196 196	131 131	154 154	222 222	0 0	153 153
M_GIR_007	94 94	113 113	80 80	106 106	129 129	92 92	78 78	130 130	223 223	150 150	196 196	131 131	154 154	222 222	326 326	153 153
M_GIR_008	94 94	113 113	80 80	106 106	129 129	92 98	78 78	130 130	223 223	150 150	196 196	131 131	154 154	222 222	326 326	153 153
M_GIR_009	94 94	113 113	80 80	106 106	129 129	92 92	78 78	130 130	223 223	150 150	196 196	131 131	154 154	222 222	326 326	153 153
M_GIR_010	94 94	113 113	80 80	106 106	131 135	0 0	78 78	130 130	223 223	150 150	196 196	131 131	154 154	222 222	326 326	153 153
M_BEN_011	0 0	0 0	88 92	0 0	125 127	78 88	0 0	114 126	0 0	152 154	208 208	119 149	162 164	224 224	336 338	145 149
M_BEN_012	0 0	0 0	78 86	0 0	105 143	88 94	0 0	114 114	0 0	150 150	208 214	119 149	158 162	224 224	334 336	145 149
M_BEN_013	122 136	113 131	86 92	102 102	125 127	92 94	86 86	114 126	223 223	150 150	208 214	119 149	158 164	224 224	324 336	145 149
M_BEN_014	0 0	0 0	86 92	0 0	125 127	92 94	0 0	114 126	0 0	150 152	208 214	119 149	158 164	224 224	324 336	145 149
M_BEN_015	0 0	0 0	86 92	0 0	125 125	92 94	0 0	114 126	0 0	150 152	208 208	125 149	158 164	224 226	334 336	149 149
M_CAM_016	0 0	0 0	80 80	0 0	137 139	78 88	0 0	128 130	0 0	150 154	208 214	125 155	158 158	222 222	324 338	149 155
M_CAM_017	0 0	0 0	80 92	0 0	139 139	78 94	0 0	114 130	0 0	152 158	214 216	125 147	158 162	224 224	324 336	149 149
M_CAM_018	0 0	0 0	80 80	0 0	137 139	88 94	0 0	126 130	0 0	154 158	214 214	125 125	158 162	222 224	324 336	149 149
M_CAM_019	110 136	111 111	80 80	92 102	137 139	78 78	86 86	126 128	225 225	158 158	208 214	119 125	158 162	222 222	338 338	149 149
M_CAM_020	0 0	0 0	80 80	0 0	137 139	78 88	0 0	128 130	0 0	150 154	208 214	125 155	158 158	222 222	324 338	149 155
M_CAM_021	0 0	0 0	80 92	0 0	139 139	78 78	0 0	114 126	0 0	154 154	210 214	119 125	158 162	222 224	326 334	149 155
M_CAM_022	0 0	0 0	92 92	0 0	139 139	78 88	0 0	126 130	0 0	150 154	210 216	119 147	158 162	222 224	334 334	149 155
M_CAM_023	0 0	0 0	80 80	0 0	139 139	78 88	0 0	114 126	0 0	150 158	214 214	125 125	158 160	222 222	334 338	145 155
M_CAM_024	0 0	0 0	80 80	0 0	137 139	78 88	0 0	126 130	0 0	152 154	208 214	119 125	158 162	222 224	324 334	149 155
M_CAM_025	0 0	0 0	92 94	0 0	133 139	78 78	0 0	114 126	0 0	154 154	214 214	125 143	156 158	220 224	324 334	145 151
M_CAM_026	0 0	0 0	80 80	0 0	137 139	78 78	0 0	124 126	0 0	150 154	210 210	153 153	156 170	220 224	324 338	145 149
M_CAM_027	0 0	0 0	86 94	0 0	125 133	78 88	0 0	124 124	0 0	150 150	214 214	123 153	158 158	224 226	324 324	145 155
M_CHD_028	0 0	0 0	0 0	0 0	105 115	78 78	0 0	130 130	0 0	0 0	196 210	133 155	164 164	222 224	324 326	147 149
M_CHD_029	0 0	0 0	0 0	0 0	115 125	78 92	0 0	126 126	0 0	0 0	214 224	119 119	156 164	220 224	324 324	149 151
M_CHD_030	0 0	0 0	0 0	0 0	133 141	78 92	0 0	112 126	0 0	0 0	196 224	119 119	158 164	224 224	324 324	147 155
M_CHD_031	0 0	0 0	0 0	0 0	139 139	78 92	0 0	126 130	0 0	0 0	214 214	125 133	160 164	220 224	324 324	145 149
M_DRC_032	0 0	0 0	76 90	0 0	133 139	92 94	0 0	126 126	0 0	150 152	206 216	143 143	166 166	222 222	330 330	149 149
M_DRC_033	0 0	0 0	88 90	0 0	125 129	92 92	0 0	114 126	0 0	150 150	216 224	125 143	156 158	222 222	324 326	145 149
M_DRC_034	0 0	0 0	80 86	0 0	131 139	88 94	0 0	126 126	0 0	150 152	214 216	137 143	156 160	220 222	326 338	149 153
M_DRC_035	0 0	0 0	80 86	0 0	127 139	86 90	0 0	114 114	0 0	152 162	210 214	119 143	158 160	220 222	324 326	149 153
M_TAN_036	102 122	111 113	80 92	100 100	123 125	0 0	76 90	114 130	213 229	0 0	0 0	0 0	0 0	0 0	0 0	0 0
M_KEN_037	0 0	0 0	80 80	0 0	105 127	88 92	0 0	122 126	0 0	154 154	196 196	141 141	154 170	216 224	324 326	147 153
M_KEN_038	0 0	0 0	80 80	0 0	105 127	92 92	0 0	122 126	0 0	154 160	196 210	141 155	154 154	216 222	324 324	147 153
M_KEN_039	0 0	0 0	80 80	0 0	127 127	92 92	0 0	130 130	0 0	160 160	196 196	119 141	154 154	216 224	324 324	147 153
M_KEN_040	0 0	0 0	80 80	0 0	125 127	84 92	0 0	114 122	0 0	154 154	210 210	119 155	170 170	216 216	324 338	145 147

B.2.b: MODERN POPULATION STR ALLELE CALLS (PAGE 2 OF 4)

ID	Leo006	Leo008	Leo085	Leo098	Leo126	Leo224	Leo230	Leo247	Leo281	FCA026	FCA032	FCA075	FCA091	FCA094	FCA208	FCA275
M_KEN_041	0 0	0 0	80 80	0 0	125 127	80 88	0 0	114 122	0 0	154 154	196 210	119 141	154 170	216 216	326 338	153 153
M_KEN_042	0 0	0 0	80 80	0 0	105 105	88 92	0 0	122 122	0 0	154 160	196 210	141 155	154 154	216 224	326 326	147 149
M_KEN_043	0 0	0 0	80 80	0 0	105 127	88 92	0 0	122 126	0 0	154 154	196 210	141 155	154 170	216 224	324 326	147 147
M_TAN_044	94 94	111 113	80 94	104 106	127 127	88 96	80 88	114 124	213 223	152 160	210 210	149 151	170 170	218 222	324 330	145 147
M_TAN_045	108 108	111 113	80 80	0 0	125 127	88 96	90 90	122 122	223 229	150 152	206 210	147 151	158 170	218 220	324 330	147 147
M_TAN_046	108 108	111 113	74 80	100 104	127 127	0 0	88 88	124 128	213 229	154 160	196 210	147 155	170 170	216 224	330 330	147 147
M_TAN_047	108 108	113 113	74 74	104 106	105 127	92 96	78 90	114 126	213 223	150 160	196 206	149 151	170 170	218 222	0 0	147 147
M_TAN_048	108 108	113 113	74 80	100 104	127 127	94 96	70 88	122 124	223 223	154 162	210 210	149 151	156 170	218 224	326 340	147 147
M_TAN_049	108 108	113 113	74 80	100 106	105 125	92 92	0 0	124 128	213 223	150 160	210 210	147 151	156 170	222 222	334 340	149 149
M_TAN_050	104 108	111 113	74 76	100 104	127 127	96 96	88 90	124 128	213 229	160 160	210 210	147 151	154 170	218 226	324 326	147 151
M_TAN_051	104 104	113 113	80 92	104 106	105 125	96 96	88 88	114 124	223 225	152 154	210 210	119 151	154 170	216 222	330 334	149 151
M_TAN_052	108 116	111 111	74 80	100 106	127 127	96 96	88 88	128 128	213 213	154 160	210 218	147 151	170 170	222 222	330 330	145 147
M_TAN_053	108 108	111 113	74 74	100 100	105 127	92 96	88 88	124 128	225 235	154 160	210 210	147 151	170 170	216 222	330 334	151 151
M_TAN_054	108 108	111 113	72 80	104 106	127 127	80 84	88 88	128 128	213 225	150 162	210 218	151 157	170 172	222 226	326 326	149 151
M_TAN_055	108 108	111 113	80 80	102 104	127 127	96 96	88 88	126 126	213 213	152 154	196 196	119 155	170 170	216 220	330 338	145 149
M_TAN_056	94 104	111 127	74 80	100 104	127 127	80 96	74 90	114 116	213 225	152 152	208 208	151 151	170 170	216 220	330 330	147 147
M_TAN_057	108 108	111 111	80 94	100 102	127 127	88 90	88 88	114 114	225 229	152 154	208 208	151 151	158 170	220 220	324 326	145 147
M_TAN_058	108 108	111 113	80 80	100 104	127 127	84 96	76 88	116 124	213 223	154 160	0 0	119 151	170 170	218 224	324 338	147 149
M_TAN_059	108 108	111 113	80 94	104 104	105 127	84 94	88 88	114 128	223 229	152 152	210 210	151 151	170 170	220 222	324 326	145 147
M_TAN_060	108 116	113 113	80 80	102 104	105 127	78 96	80 88	116 124	213 229	150 154	210 218	149 151	170 170	222 224	326 330	147 149
M_TAN_061	108 116	111 127	80 80	100 104	127 129	80 94	74 88	122 124	213 235	154 160	208 210	119 147	170 170	216 224	326 330	145 145
M_TAN_062	108 108	0 0	80 80	102 102	127 141	80 92	80 90	122 130	213 229	152 154	206 208	137 147	170 170	216 220	326 330	145 147
M_TAN_063	94 108	111 111	80 80	100 104	105 127	96 96	88 88	124 128	213 223	154 154	210 210	149 151	170 170	220 224	326 326	145 147
M_ZAM_064	108 124	111 127	80 80	102 102	125 125	78 92	88 88	126 128	213 217	0 0	0 0	0 0	0 0	0 0	0 0	0 0
M_ZAM_065	124 124	111 111	80 94	102 102	127 127	86 86	0 0	114 116	213 223	160 160	206 206	137 143	174 174	220 220	330 330	145 149
M_ZAM_066	108 108	111 111	74 80	92 102	125 125	78 86	0 0	126 126	213 217	162 164	216 216	119 147	162 162	218 218	330 330	145 149
M_ZAM_067	108 122	111 113	80 80	102 106	125 127	88 92	76 76	122 128	213 213	150 154	206 206	147 149	158 174	220 224	332 332	149 151
M_ZAM_068	124 124	111 113	80 94	102 106	125 125	78 92	76 88	126 128	213 213	150 150	208 208	137 155	170 174	222 222	318 328	149 149
M_ZAM_069	104 108	111 125	80 80	102 102	125 135	86 92	78 92	114 116	229 251	150 150	206 206	0 0	170 174	220 224	326 336	151 151
M_ZAM_070	104 108	111 125	80 80	102 102	125 127	86 92	76 88	114 126	213 229	152 152	206 216	147 159	168 170	222 222	324 324	149 149
M_ZAM_071	108 124	113 113	74 74	102 104	125 125	78 92	80 88	126 126	213 243	0 0	0 0	0 0	0 0	0 0	0 0	0 0
M_ZAM_072	124 124	111 113	80 80	102 102	125 127	86 94	88 88	128 128	213 233	150 160	206 218	147 151	154 168	222 222	330 336	149 151
M_ZAM_073	108 124	113 113	74 80	102 102	127 127	78 86	88 88	114 128	213 217	162 206	216 179	157 135	174 224	224 159	328 140	149 149
M_ZAM_074	104 108	111 111	76 80	102 106	125 131	78 86	80 88	128 128	213 217	0 0	0 0	0 0	0 0	0 0	0 0	0 0
M_ZAM_075	108 122	111 113	80 80	102 106	135 143	86 92	84 90	116 128	213 213	0 0	0 0	0 0	0 0	0 0	0 0	0 0
M_ZAM_076	94 104	111 113	80 80	102 106	125 131	78 78	80 88	116 128	213 223	0 0	0 0	0 0	0 0	0 0	0 0	0 0
M_ZAM_077	108 124	111 127	80 94	102 104	125 127	86 86	88 90	124 128	213 253	0 0	0 0	0 0	0 0	0 0	0 0	0 0
M_ZAM_078	82 124	113 113	76 94	102 102	105 129	86 86	0 0	124 132	217 245	154 206	210 179	149 139	170 216	222 159	330 136	149 151
M_ZAM_079	122 122	113 123	74 94	104 104	105 129	78 94	76 80	116 126	213 233	0 0	0 0	0 0	0 0	0 0	0 0	0 0
M_ZAM_080	96 122	127 131	74 80	102 104	125 125	86 86	76 82	128 132	217 247	0 0	0 0	0 0	0 0	0 0	0 0	0 0

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ID	Leo006	Leo008	Leo085	Leo098	Leo126	Leo224	Leo230	Leo247	Leo281	FCA026	FCA032	FCA075	FCA091	FCA094	FCA208	FCA275
M_ZAM_081	104 108	131 131	94 94	104 104	105 127	86 94	82 88	122 128	211 247	0 0	0 0	0 0	0 0	0 0	0 0	0 0
M_ZAM_082	104 122	113 127	74 94	104 104	105 127	78 92	76 88	128 128	213 233	0 0	0 0	0 0	0 0	0 0	0 0	0 0
M_ZAM_083	94 110	111 131	80 80	104 106	125 127	78 86	76 80	128 128	245 245	0 0	0 0	0 0	0 0	0 0	0 0	0 0
M_ZAM_084	94 96	111 131	74 80	92 104	125 127	78 86	76 80	126 128	217 233	0 0	0 0	0 0	0 0	0 0	0 0	0 0
M_ZAM_085	104 122	111 131	80 80	106 106	125 127	78 92	76 82	128 128	213 247	0 0	0 0	0 0	0 0	0 0	0 0	0 0
M_ZAM_086	104 110	127 131	86 94	94 102	127 127	86 94	80 80	126 128	229 233	0 0	0 0	0 0	0 0	0 0	0 0	0 0
M_ZAM_087	96 108	113 131	80 86	104 104	125 127	86 94	80 80	116 128	245 245	0 0	0 0	0 0	0 0	0 0	0 0	0 0
M_ZAM_088	96 122	113 127	74 80	104 108	127 127	86 86	74 82	114 116	213 213	0 0	0 0	0 0	0 0	0 0	0 0	0 0
M_ZAM_089	104 110	127 131	74 74	102 106	125 127	78 86	76 82	128 128	213 233	0 0	0 0	0 0	0 0	0 0	0 0	0 0
M_ZAM_090	122 122	111 131	76 80	106 108	105 125	86 92	74 80	124 126	211 215	0 0	0 0	0 0	0 0	0 0	0 0	0 0
M_RSA_091	104 108	113 127	80 86	104 106	105 125	88 96	82 84	122 128	211 223	0 0	0 0	0 0	0 0	0 0	0 0	0 0
M_RSA_092	122 124	113 127	80 94	94 104	105 123	96 96	90 90	114 122	233 237	0 0	0 0	0 0	0 0	0 0	0 0	0 0
M_RSA_093	94 122	113 127	92 92	106 110	127 139	78 96	80 90	122 124	213 229	0 0	0 0	0 0	0 0	0 0	0 0	0 0
M_ZAM_094	122 124	111 127	80 80	102 104	127 143	96 96	90 92	126 128	213 213	0 0	0 0	0 0	0 0	0 0	0 0	0 0
M_RSA_095	94 108	111 127	80 80	102 104	105 105	96 96	80 90	114 124	233 233	154 160	206 208	153 153	162 170	218 222	330 330	151 151
M_RSA_096	104 104	111 127	74 94	100 104	125 125	78 92	88 90	128 136	217 239	160 162	210 210	141 145	170 170	220 224	336 336	147 151
M_RSA_097	104 104	111 127	80 80	102 104	127 129	80 84	88 88	114 122	229 229	154 160	206 216	147 147	164 170	220 224	332 336	151 151
M_RSA_098	94 108	127 127	76 80	104 108	123 125	80 96	82 90	114 122	213 229	154 160	208 216	141 153	170 174	220 220	332 338	151 151
M_RSA_099	104 108	111 111	80 94	100 102	105 129	90 96	88 90	114 124	233 233	154 160	206 210	145 145	162 170	220 224	332 336	147 149
M_RSA_100	110 122	111 127	80 80	100 102	123 143	78 86	80 88	114 136	211 229	160 160	206 208	147 153	162 170	218 220	332 336	151 151
M_RSA_101	104 108	111 127	80 80	104 104	123 125	78 96	82 90	136 136	213 233	154 160	208 216	147 147	168 170	218 224	336 336	147 151
M_RSA_102	104 108	111 129	80 94	102 106	127 129	92 96	88 88	114 124	229 245	154 162	210 216	141 153	162 174	220 222	332 336	149 151
M_RSA_103	112 124	111 127	80 94	104 104	125 127	80 92	82 84	114 114	225 229	154 154	210 212	147 153	168 170	224 224	332 338	151 151
M_RSA_104	108 122	111 127	76 80	106 106	125 143	92 96	88 94	114 114	239 245	154 160	206 216	145 149	162 162	216 220	336 336	147 151
M_RSA_105	94 110	111 127	80 80	94 104	127 127	96 96	80 88	114 122	213 247	150 160	206 206	141 149	162 164	216 220	330 336	151 151
M_RSA_106	122 124	111 113	74 76	92 102	127 129	78 94	82 90	114 122	213 213	150 150	206 210	119 141	164 164	222 222	324 336	151 151
M_RSA_107	110 122	111 113	80 80	94 94	127 127	78 92	76 88	114 122	211 213	154 160	210 216	141 149	166 172	218 220	324 332	145 147
M_RSA_108	104 122	111 113	80 80	94 104	127 127	78 96	88 90	116 128	213 223	154 154	206 216	119 141	162 164	218 222	324 324	151 151
M_RSA_109	96 124	111 127	74 76	102 102	127 127	78 94	80 82	124 128	213 213	150 160	208 210	141 151	162 172	218 226	324 334	145 151
M_RSA_110	110 124	113 127	76 76	94 102	127 127	78 94	82 82	122 124	215 215	154 160	216 216	119 149	164 164	218 226	324 336	147 147
M_RSA_111	104 124	111 113	76 80	94 102	127 127	78 92	76 76	116 116	213 213	150 160	206 210	119 151	164 164	216 222	324 330	149 149
M_RSA_112	110 124	111 113	74 80	94 104	127 127	78 92	76 88	122 122	213 215	160 160	210 210	141 151	178 178	218 220	330 336	147 151
M_RSA_113	110 124	111 127	80 80	104 104	129 129	78 96	88 88	116 124	213 223	150 150	206 210	141 141	162 166	216 218	0 0	147 151
M_RSA_114	94 124	113 127	76 80	94 104	127 129	92 94	82 88	122 122	211 211	150 160	210 216	149 151	164 166	216 218	336 336	145 147
M_ZAM_115	96 130	131 131	74 76	102 108	127 129	78 78	76 80	114 128	213 213	0 0	0 0	0 0	0 0	0 0	0 0	0 0
M_NAM_116	94 104	111 113	0 0	102 108	129 129	78 78	76 76	128 128	229 229	150 154	0 0	119 141	170 170	220 224	324 324	151 153
M_NAM_117	0 0	113 127	76 80	102 106	105 129	86 86	76 80	128 128	213 213	154 160	196 216	147 151	164 170	218 220	324 334	149 149
M_NAM_118	110 132	127 127	76 76	102 112	105 129	86 94	80 80	114 128	213 213	150 160	216 216	141 151	164 170	218 220	334 336	149 151
M_NAM_119	110 122	127 131	76 76	104 108	129 129	86 94	80 82	114 114	213 213	160 160	216 216	141 151	168 174	218 224	324 324	149 151
M_NAM_120	132 132	111 111	76 76	102 102	127 129	78 84	76 82	114 128	213 213	160 160	216 216	141 141	162 168	218 224	324 336	149 151

B.2.b: MODERN POPULATION STR ALLELE CALLS (PAGE 4 OF 4)

ID	Leo006	Leo008	Leo085	Leo098	Leo126	Leo224	Leo230	Leo247	Leo281	FCA026	FCA032	FCA075	FCA091	FCA094	FCA208	FCA275
M_NAM_121	94 110	127 127	76 80	102 112	129 129	78 92	76 76	114 128	229 229	150 160	216 216	141 151	170 170	224 224	334 336	149 149
M_NAM_122	0 0	111 127	76 80	112 112	129 129	78 94	76 76	114 128	213 229	154 160	206 216	149 151	164 170	218 224	324 324	149 149
M_NAM_123	94 132	111 127	76 76	104 108	105 129	78 94	76 82	114 128	213 223	154 160	216 216	149 149	164 168	220 220	334 334	151 151
M_NAM_124	104 132	111 127	76 94	108 112	129 129	94 94	76 82	114 128	213 229	160 160	206 210	141 149	164 174	218 220	324 338	149 149
M_NAM_125	110 132	111 133	76 80	102 108	105 129	94 94	80 82	114 114	213 229	150 160	196 216	141 147	168 170	220 224	334 334	149 151
M_ZAM_126	104 122	127 131	76 80	102 102	127 129	92 92	76 76	114 122	229 233	0 0	0 0	0 0	0 0	0 0	0 0	0 0
M_ZAM_127	108 128	111 111	80 80	102 106	105 127	86 86	76 90	116 124	217 245	0 0	0 0	0 0	0 0	0 0	0 0	0 0
M_ZAM_128	94 124	127 131	76 80	102 104	105 127	78 86	76 88	122 126	213 247	0 0	0 0	0 0	0 0	0 0	0 0	0 0
M_ZAM_129	94 108	113 131	76 80	102 104	125 129	86 94	76 86	114 116	213 213	0 0	0 0	0 0	0 0	0 0	0 0	0 0
M_RSA_130	122 130	113 127	74 80	104 108	125 129	86 96	0 0	114 116	213 229	0 0	0 0	0 0	0 0	0 0	0 0	0 0
M_ZAM_131	94 94	127 131	74 94	102 104	129 129	78 94	84 90	116 122	213 213	0 0	0 0	0 0	0 0	0 0	0 0	0 0
M_DRC_132	0 0	0 0	76 94	0 0	127 129	78 92	0 0	122 122	0 0	150 152	206 208	143 143	166 170	222 222	330 340	149 151
M_DRC_133	0 0	0 0	92 94	0 0	129 129	86 92	0 0	126 126	0 0	150 152	216 218	143 145	156 170	222 222	326 340	149 151
M_DRC_134	0 0	0 0	80 90	0 0	129 147	84 98	0 0	114 122	0 0	150 150	210 214	143 151	170 174	220 220	326 334	149 153
M_TAN_135	124 124	113 125	76 80	102 102	129 143	88 96	76 88	114 116	213 223	0 0	0 0	0 0	0 0	0 0	0 0	0 0

B.2.c: Historical Population STR Allele Calls (5 Pages)

ID	Leo006	Leo008	Leo031	Leo045	Leo077	Leo085	Leo098	Leo126	Leo224	Leo230	Leo247	Leo281	Leo391	Leo506
H_BOT_001	94 104	129 129	0 0	80 80	96 102	80 80	102 102	127 127	78 86	0 0	120 128	0 0	0 0	0 0
H_BOT_002	104 122	127 127	192 196	80 94	104 104	80 80	102 102	105 127	78 96	78 90	122 128	211 229	174 174	171 193
H_COG_003	94 94	111 129	196 196	80 80	104 104	80 94	100 102	125 125	78 86	86 86	114 128	213 213	174 186	0 0
H_GAB_004	108 122	111 131	196 196	80 80	104 106	80 80	102 104	105 125	78 94	88 88	122 122	213 213	186 198	171 195
H_CPT_005	0 0	131 131	192 196	0 0	0 0	76 92	104 106	111 121	90 90	80 80	122 126	0 0	0 0	0 0
H_CPT_006	108 124	113 127	190 196	80 90	102 102	76 92	104 108	105 125	92 98	80 84	122 122	205 213	170 182	191 191
H_CPT_007	126 126	119 131	0 0	76 76	98 102	76 76	100 112	119 135	86 86	78 78	122 122	185 231	0 0	0 0
H_CPT_008	0 0	0 0	0 0	0 0	0 0	78 78	102 102	0 0	86 86	88 88	110 110	0 0	0 0	195 195
H_CPT_009	100 100	129 129	172 192	104 104	98 98	84 94	102 106	121 121	84 90	88 88	114 114	229 233	166 182	193 197
H_ZIM_010	94 108	113 127	196 196	80 80	104 108	76 80	102 102	125 127	78 92	80 88	114 128	213 217	182 190	171 193
H_MWI_011	104 124	125 131	192 196	78 80	98 104	80 94	102 102	105 123	78 86	90 90	116 126	0 0	190 190	0 0
H_COG_012	90 104	129 129	196 196	0 0	104 104	80 94	92 104	0 0	86 86	88 88	114 122	0 0	0 0	0 0
H_COG_013	94 108	113 131	196 196	80 80	104 106	94 94	102 106	125 135	86 96	76 80	114 126	213 213	174 186	173 193
H_NAM_014	104 124	113 125	196 196	80 80	0 0	76 86	92 104	125 125	84 90	86 90	114 122	213 213	0 0	0 0
H_CPT_015	94 110	113 129	0 0	80 80	98 102	76 92	94 104	125 139	78 86	90 90	118 132	213 213	174 178	0 0
H_KEN_016	124 124	111 121	0 0	80 100	104 106	76 80	102 102	125 127	78 96	0 0	126 130	0 0	174 182	193 193
H_RSA_017	0 0	111 129	196 196	0 0	98 98	78 78	102 102	117 121	86 86	80 80	120 120	0 0	190 190	0 0
H_KEN_018	132 132	129 129	0 0	106 110	92 94	80 94	104 108	127 143	86 86	80 80	112 124	211 211	0 0	171 173
H_KEN_019	82 94	129 129	0 0	0 0	98 110	80 80	102 104	125 129	86 86	80 80	114 122	0 0	174 174	0 0
H_KEN_020	94 94	111 129	194 194	80 80	104 104	80 92	102 104	123 127	78 86	76 88	114 120	209 209	178 186	0 0
H_KEN_021	82 124	111 129	190 192	92 92	102 104	80 80	106 110	105 105	86 96	80 88	114 126	217 217	170 190	0 0
H_KEN_022	94 122	111 129	186 194	80 80	98 110	80 80	104 104	127 127	92 94	76 88	114 116	213 223	174 186	171 195
H_KEN_023	124 128	111 129	196 196	80 108	102 108	76 80	102 104	105 129	96 96	78 90	114 126	211 235	170 186	0 0
H_KEN_024	104 104	131 131	0 0	80 80	0 0	78 94	102 102	125 127	86 86	80 80	116 120	211 229	0 0	0 0
H_KEN_025	94 124	131 131	198 198	80 80	104 104	76 94	104 108	105 129	86 86	86 88	116 116	0 0	0 0	0 0
H_KEN_026	94 104	111 125	186 196	100 108	110 110	80 80	104 108	125 125	96 96	76 80	122 128	213 213	174 190	187 193
H_CPT_027	82 82	131 131	176 194	80 80	106 106	80 92	92 100	105 111	92 92	88 88	106 106	227 229	190 190	0 0
H_KEN_028	122 124	111 129	186 194	80 108	108 110	80 80	102 104	127 127	84 96	78 84	116 128	213 223	174 174	171 171
H_KEN_029	104 124	113 125	192 196	78 78	0 0	78 94	0 0	121 125	84 84	76 80	122 122	211 211	0 0	0 0
H_CPT_030	104 134	0 0	196 196	78 80	0 0	0 0	92 98	0 0	84 86	80 80	114 122	211 211	0 0	211 211
H_DRC_031	110 132	113 129	196 196	80 94	98 106	76 76	104 110	133 133	86 90	76 86	124 124	213 225	170 182	0 0
H_DRC_032	122 124	129 129	196 196	80 80	98 98	78 94	100 102	121 121	78 86	76 76	126 126	229 229	194 194	0 0
H_DRC_033	110 110	129 129	194 194	80 80	98 108	92 92	104 110	127 127	86 86	78 80	122 126	213 229	0 0	195 195
H_DRC_034	94 104	111 129	184 186	78 80	110 110	80 80	106 110	125 125	96 96	80 86	122 130	213 213	174 174	0 0
H_DRC_035	92 92	129 129	0 0	80 80	98 98	78 78	0 0	129 129	80 86	80 80	114 114	211 211	0 0	0 0
H_DRC_036	0 0	113 129	0 0	0 0	100 102	80 92	100 112	0 0	92 92	80 80	118 118	0 0	178 182	0 0
H_DRC_037	92 104	127 129	0 0	80 102	0 0	0 0	102 102	127 131	88 88	78 78	122 122	211 225	0 0	0 0

B.2.c: HISTORICAL POPULATION STR ALLELE CALLS (PAGE 2 OF 5)

ID	Leo006	Leo008	Leo031	Leo045	Leo077	Leo085	Leo098	Leo126	Leo224	Leo230	Leo247	Leo281	Leo391	Leo506
H_DRC_038	104 120	113 125	192 196	80 80	96 96	78 94	104 104	125 125	78 86	76 80	114 122	211 229	0 0	193 197
H_DRC_039	110 110	127 131	186 196	82 82	108 110	76 80	104 108	125 131	86 90	76 84	122 124	229 229	178 182	187 189
H_DRC_040	108 108	113 129	196 196	80 80	98 120	76 86	102 108	125 129	86 86	84 84	116 122	207 211	178 178	0 0
H_DRC_041	104 104	111 129	196 196	78 80	0 0	80 92	94 104	125 125	86 88	0 0	114 122	0 0	178 182	0 0
H_DRC_042	110 130	111 131	194 196	80 80	100 110	92 92	92 112	125 147	92 96	76 86	122 124	223 229	178 182	171 175
H_DRC_043	94 132	113 125	0 0	80 80	96 102	78 94	102 108	125 133	90 90	82 82	122 122	209 227	0 0	0 0
H_KEN_044	82 110	111 131	186 196	82 82	100 100	80 92	104 108	125 125	88 94	78 84	122 122	213 223	170 174	171 171
H_KEN_045	82 110	111 129	186 186	80 80	100 100	80 80	108 108	123 125	70 94	78 78	122 126	213 223	174 178	171 171
H_KEN_046	82 118	111 129	186 186	70 98	100 100	80 80	108 108	125 125	92 92	78 86	122 126	211 211	174 198	171 193
H_KEN_047	112 112	127 127	182 182	102 102	104 104	96 96	104 104	133 135	84 98	88 88	108 108	197 199	0 0	197 197
H_KEN_048	104 106	129 129	182 196	108 108	104 106	98 98	104 106	135 135	84 98	88 88	108 108	197 229	162 170	173 183
H_KEN_049	104 106	123 123	182 196	104 104	100 104	98 98	106 108	135 141	78 84	80 94	102 108	197 199	0 0	209 211
H_GIR_050	94 94	129 129	192 192	80 80	98 98	78 78	92 104	0 0	86 86	80 80	124 128	229 229	0 0	0 0
H_GIR_051	94 94	113 129	0 0	80 110	106 110	80 80	104 106	127 127	86 90	80 80	122 130	209 229	0 0	0 0
H_CPT_052	94 122	111 127	192 194	80 86	102 108	76 92	106 108	105 139	78 80	80 90	114 124	213 223	162 174	171 171
H_CPT_053	128 128	113 123	192 192	80 80	106 106	76 88	102 110	125 143	90 90	78 84	114 126	213 213	178 190	175 175
H_GIR_054	94 94	113 125	192 192	80 80	106 106	80 80	106 106	127 129	92 92	78 78	130 130	223 223	186 186	171 175
H_CPT_055	104 104	111 129	196 196	80 80	102 102	76 80	106 106	105 105	78 92	80 80	114 128	213 213	182 182	0 0
H_CPT_056	122 124	127 129	192 192	80 80	98 98	78 78	102 102	125 125	86 86	0 0	120 120	209 213	190 190	0 0
H_KEN_057	82 108	113 117	186 194	80 80	100 106	92 92	102 108	125 125	86 86	78 88	114 122	213 223	166 182	0 0
H_ANG_058	130 130	111 129	196 196	104 104	102 106	76 80	92 102	105 129	78 90	76 80	114 114	213 223	174 186	171 171
H_RSA_059	108 108	111 127	192 196	80 80	102 108	74 80	106 106	105 125	96 96	88 88	114 122	211 233	182 186	0 0
H_RSA_060	104 110	111 127	192 192	0 0	102 102	74 80	104 104	123 123	86 92	92 92	114 132	213 213	170 182	195 195
H_RSA_061	122 124	131 131	192 192	80 80	106 106	80 80	106 106	129 129	86 86	80 80	0 0	213 229	0 0	0 0
H_RSA_062	94 110	111 127	192 192	80 80	102 102	74 80	92 104	125 127	78 96	84 90	116 124	213 235	182 182	159 171
H_RSA_063	104 104	113 129	196 196	78 80	104 108	74 80	98 102	127 129	78 78	0 0	114 122	213 229	0 0	0 0
H_RSA_064	94 104	111 125	196 196	80 80	102 104	76 80	104 106	105 125	86 92	80 90	114 122	213 229	170 182	0 0
H_RSA_065	108 122	113 125	192 196	80 80	102 108	80 80	104 108	105 125	86 92	82 92	114 122	213 235	186 190	171 171
H_RSA_066	108 108	131 131	192 196	80 80	106 106	74 80	106 106	123 123	78 86	80 88	122 136	213 229	182 182	0 0
H_CAR_067	94 110	111 129	186 194	80 80	100 100	80 86	104 108	137 137	78 90	80 86	126 130	225 225	170 186	171 171
H_CPT_068	110 112	119 119	176 176	74 78	106 106	82 90	104 104	101 143	90 90	78 78	120 120	211 219	0 0	0 0
H_BOT_069	94 104	127 129	192 196	106 106	104 104	80 80	102 102	105 127	78 92	82 82	122 128	213 213	174 190	0 0
H_BOT_070	104 122	123 127	192 196	80 94	104 106	80 80	104 104	129 129	86 92	78 90	122 132	211 213	174 174	171 195
H_BOT_071	122 122	111 131	192 196	80 80	104 106	76 80	104 104	127 129	86 90	76 80	116 128	213 213	174 178	173 193
H_BOT_072	0 0	129 129	0 0	0 0	0 0	78 94	100 100	0 0	78 78	78 80	118 120	0 0	0 0	0 0
H_BOT_073	122 122	127 127	192 196	100 106	102 104	76 80	102 102	105 129	78 96	80 88	128 128	211 211	174 194	171 191
H_BOT_074	94 122	129 129	192 196	80 80	102 104	80 80	102 102	125 129	78 92	78 78	122 122	211 229	174 194	171 189

B.2.c: HISTORICAL POPULATION STR ALLELE CALLS (PAGE 3 OF 5)

ID	Leo006	Leo008	Leo031	Leo045	Leo077	Leo085	Leo098	Leo126	Leo224	Leo230	Leo247	Leo281	Leo391	Leo506
H_BOT_075	96 122	113 131	188 192	80 80	104 104	76 76	102 102	105 129	78 92	76 76	128 128	213 227	174 174	171 171
H_BOT_076	96 122	113 127	192 192	80 94	102 102	76 80	104 106	127 127	78 92	78 88	122 128	213 213	174 174	171 191
H_BOT_077	96 122	113 129	196 196	78 80	102 102	76 80	92 104	127 129	78 92	0 0	114 122	209 213	174 194	0 0
H_CPT_078	82 98	129 129	0 0	76 98	100 100	78 94	98 102	0 0	84 86	78 78	120 126	185 229	170 170	0 0
H_TAN_079	114 114	125 131	184 184	72 72	96 98	70 94	104 106	111 111	80 80	80 80	122 122	211 211	0 0	0 0
H_TAN_080	96 120	111 129	186 186	78 80	110 110	80 92	100 104	105 127	80 86	80 80	114 130	211 213	178 182	0 0
H_TAN_081	124 128	113 113	196 196	80 94	106 108	74 80	100 102	125 127	80 88	76 84	122 124	225 229	174 178	195 195
H_TAN_082	104 104	129 129	192 196	78 80	108 108	78 78	0 0	127 129	86 86	78 80	0 0	213 227	0 0	0 0
H_TAN_083	112 122	113 113	184 196	80 94	102 104	74 74	100 106	127 127	86 88	76 76	114 122	213 223	174 186	173 195
H_TAN_084	94 124	111 129	196 196	80 80	102 106	80 92	100 102	105 127	92 96	80 88	122 122	225 235	178 186	171 171
H_TAN_085	124 132	113 129	184 196	80 102	98 102	80 80	100 100	105 105	80 96	76 80	126 126	213 223	0 0	0 0
H_TAN_086	120 122	125 129	196 196	80 80	102 110	80 94	102 104	123 127	84 86	0 0	110 126	211 211	170 170	0 0
H_TAN_087	122 124	111 111	196 196	80 94	102 110	80 94	104 106	105 125	88 96	84 84	122 130	213 213	174 198	173 193
H_TAN_088	0 0	113 131	0 0	0 0	106 108	74 80	100 102	0 0	80 86	76 84	114 122	0 0	190 190	0 0
H_TAN_089	94 96	111 111	186 196	80 80	104 108	76 92	104 104	127 127	88 94	76 90	128 128	223 223	178 178	173 195
H_TAN_090	96 122	111 111	186 196	80 94	104 110	80 92	104 106	105 143	88 96	78 84	122 134	213 223	170 190	187 193
H_KEN_091	106 124	111 129	196 196	80 80	102 102	80 92	108 108	125 127	88 96	74 82	122 130	207 223	170 174	171 171
H_KEN_092	94 112	113 125	196 196	0 0	106 110	80 94	102 102	0 0	90 90	74 74	122 130	0 0	0 0	0 0
H_KEN_093	82 112	113 129	188 196	80 80	102 104	80 92	102 102	0 0	88 96	82 90	114 122	213 229	174 182	0 0
H_KEN_094	112 124	111 129	192 196	80 80	100 104	92 94	108 108	105 127	84 94	80 88	122 126	213 229	174 178	0 0
H_KEN_095	110 124	113 125	196 196	80 102	102 104	80 92	92 104	105 125	88 88	74 78	114 122	213 223	170 174	171 193
H_KEN_096	94 124	111 129	186 196	78 102	102 104	80 92	102 108	125 127	78 88	80 80	122 130	223 227	174 194	193 193
H_CPT_097	94 94	129 129	196 196	80 80	0 0	78 92	100 104	129 129	84 86	0 0	114 120	0 0	0 0	0 0
H_CPT_098	94 110	113 129	192 196	80 80	102 104	80 92	100 108	125 143	78 78	80 80	114 114	213 225	174 178	0 0
H_CPT_099	110 110	113 127	0 0	80 80	98 110	80 92	102 110	139 143	78 90	82 82	114 124	225 225	174 178	0 0
H_CPT_100	90 90	0 0	0 0	110 110	0 0	80 80	102 102	105 105	92 92	0 0	118 118	0 0	0 0	0 0
H_UNK_101	92 92	113 129	194 194	0 0	0 0	92 92	0 0	113 113	86 86	0 0	0 0	0 0	0 0	0 0
H_UNK_102	0 0	113 129	0 0	0 0	102 102	94 94	100 106	0 0	0 0	90 90	0 0	0 0	0 0	0 0
H_UNK_103	90 90	113 127	184 186	110 110	98 106	80 92	102 102	105 113	84 92	76 80	122 124	185 221	174 194	0 0
H_ETH_104	124 124	129 129	0 0	78 78	0 0	78 84	104 104	127 139	86 94	0 0	122 122	0 0	0 0	0 0
H_TAN_105	124 124	125 129	186 186	80 94	108 110	80 80	102 102	127 127	92 96	0 0	114 126	185 221	174 174	0 0
H_TAN_106	104 108	129 129	0 0	78 80	98 98	80 80	102 108	129 129	92 92	0 0	122 126	213 213	0 0	0 0
H_TAN_107	90 112	111 127	186 196	80 94	102 110	80 92	102 104	127 127	86 96	86 90	126 130	207 213	186 198	173 175
H_TAN_108	128 130	125 129	0 0	78 80	98 98	80 94	100 100	105 129	86 88	0 0	126 126	0 0	0 0	0 0
H_TAN_109	94 94	0 0	0 0	78 80	0 0	0 0	102 102	121 121	92 92	0 0	126 126	0 0	190 190	0 0
H_TAN_110	122 122	111 129	196 196	80 80	98 98	0 0	100 100	105 129	82 82	90 90	122 122	0 0	0 0	0 0
H_TAN_111	96 96	113 127	0 0	80 80	0 0	0 0	100 100	105 129	86 86	0 0	0 0	0 0	0 0	0 0

B.2.c: HISTORICAL POPULATION STR ALLELE CALLS (PAGE 4 OF 5)

ID	Leo006	Leo008	Leo031	Leo045	Leo077	Leo085	Leo098	Leo126	Leo224	Leo230	Leo247	Leo281	Leo391	Leo506
H_TAN_112	0 0	125 129	0 0	0 0	0 0	76 92	104 104	0 0	86 86	0 0	122 122	0 0	0 0	0 0
H_SOM_113	110 110	113 127	0 0	78 80	0 0	78 78	100 108	105 129	84 96	0 0	116 122	0 0	0 0	0 0
H_KEN_114	0 0	127 129	0 0	80 80	98 98	78 78	100 100	121 129	84 86	0 0	122 122	0 0	178 178	0 0
H_KEN_115	124 124	111 127	186 186	80 80	102 106	80 86	102 108	125 127	80 96	80 80	114 126	223 223	182 186	193 195
H_KEN_116	0 0	127 127	0 0	78 80	98 102	80 80	102 102	103 103	86 86	0 0	122 124	0 0	0 0	0 0
H_KEN_117	110 110	129 129	0 0	78 78	98 102	80 80	102 102	127 127	80 92	0 0	114 122	0 0	182 182	0 0
H_KEN_118	94 94	113 127	196 196	80 80	104 110	74 92	102 102	105 129	74 88	76 76	114 126	0 0	182 182	0 0
H_SDN_119	120 120	113 129	0 0	80 108	0 0	78 78	102 102	121 129	84 84	80 80	0 0	211 229	0 0	0 0
H_GIR_120	94 94	113 125	192 192	80 80	106 106	80 80	106 106	129 129	86 92	78 78	130 130	223 223	186 186	171 175
H_TAN_121	0 0	0 0	0 0	0 0	0 0	0 0	102 104	0 0	0 0	0 0	0 0	0 0	0 0	0 0
H_TAN_122	0 0	113 127	196 196	78 80	100 100	76 92	106 110	123 129	84 86	80 80	128 128	0 0	0 0	0 0
H_TAN_123	0 0	115 129	0 0	78 80	0 0	80 80	100 102	105 129	88 88	0 0	114 120	0 0	0 0	0 0
H_TAN_124	0 0	0 0	186 188	0 0	0 0	0 0	98 98	0 0	76 92	0 0	118 118	0 0	0 0	0 0
H_TAN_125	94 112	111 111	192 194	80 80	102 102	74 80	102 102	105 105	80 96	84 88	122 122	223 235	174 178	195 195
H_TAN_126	94 112	113 129	192 194	80 94	102 102	80 80	104 104	105 143	88 96	84 88	122 122	223 223	174 182	193 193
H_BOT_127	94 94	113 129	192 192	80 80	102 106	74 80	96 104	127 127	78 82	0 0	122 136	223 223	174 174	0 0
H_BOT_128	0 0	113 129	192 196	80 80	0 0	0 0	102 102	121 121	84 84	0 0	112 122	229 229	0 0	0 0
H_BOT_129	96 96	111 127	192 192	80 80	104 104	80 80	102 104	127 127	78 86	78 86	122 128	0 0	0 0	193 193
H_BOT_130	96 96	111 127	0 0	78 80	104 106	76 80	102 102	127 129	78 92	78 78	124 128	0 0	0 0	0 0
H_BOT_131	0 0	0 0	186 186	78 80	0 0	80 80	100 104	127 129	84 92	0 0	112 124	211 219	0 0	0 0
H_RSA_132	104 110	113 127	188 196	80 94	102 104	96 96	104 106	105 125	84 96	76 88	114 122	215 223	174 186	171 191
H_BOT_133	96 122	127 131	192 196	94 100	102 104	76 80	102 102	129 129	86 92	80 86	124 128	213 229	174 194	171 193
H_MLI_134	102 104	113 127	182 182	78 80	0 0	76 92	104 104	119 129	86 86	0 0	122 122	213 229	0 0	0 0
H_KEN_135	0 0	0 0	0 0	0 0	0 0	76 76	0 0	0 0	84 84	0 0	122 122	0 0	0 0	0 0
H_KEN_136	0 0	113 127	0 0	0 0	0 0	78 76	104 108	0 0	84 86	90 90	122 126	0 0	178 194	0 0
H_BOT_137	94 120	113 127	192 196	80 80	104 106	74 80	102 104	125 127	86 92	78 86	122 122	213 217	174 190	171 171
H_SDN_138	0 0	0 0	0 0	0 0	0 0	78 78	0 0	0 0	0 0	0 0	116 120	0 0	0 0	0 0
H_ZAM_139	122 122	111 127	190 190	80 80	100 102	76 92	104 104	105 105	86 86	0 0	116 128	211 227	174 174	0 0
H_TAN_140	104 104	125 131	192 192	78 78	96 96	94 94	102 102	0 0	86 86	0 0	122 122	213 229	178 178	0 0
H_TAN_141	82 130	111 129	186 196	80 80	102 104	80 92	100 102	127 129	92 96	78 84	116 130	221 225	170 186	187 195
H_KEN_142	96 122	111 131	184 192	80 80	98 102	74 80	100 102	125 129	80 94	78 90	122 128	229 241	178 190	0 0
H_KEN_143	90 122	113 113	196 196	80 110	104 106	94 94	104 104	105 105	94 94	80 90	128 128	223 229	174 190	171 175
H_KEN_144	92 92	0 0	0 0	80 80	0 0	0 0	104 104	123 129	0 0	0 0	0 0	213 213	0 0	0 0
H_TAN_145	0 0	125 131	198 198	0 0	96 102	78 80	102 102	0 0	86 86	82 82	128 128	233 233	0 0	0 0
H_ANG_146	92 104	131 131	0 0	78 80	96 96	0 0	108 108	121 127	86 86	90 90	112 122	211 227	0 0	0 0
H_NRT_147	82 104	0 0	0 0	0 0	98 106	0 0	108 112	129 129	84 88	0 0	118 120	213 217	0 0	0 0
H_MOZ_148	94 94	103 129	0 0	92 92	98 98	0 0	98 98	0 0	0 0	0 0	0 0	0 0	0 0	0 0

B.2.c: HISTORICAL POPULATION STR ALLELE CALLS (PAGE 5 OF 5)

ID	Leo006	Leo008	Leo031	Leo045	Leo077	Leo085	Leo098	Leo126	Leo224	Leo230	Leo247	Leo281	Leo391	Leo506
H_MOZ_149	122 122	0 0	0 0	78 92	96 102	92 92	102 108	105 127	86 90	0 0	0 0	211 211	0 0	0 0
H_NAM_150	94 94	111 127	196 196	80 80	102 106	74 76	92 98	105 129	86 86	90 90	116 122	213 223	186 190	0 0
H_NRT_151	0 0	0 0	0 0	80 80	0 0	0 0	102 102	125 129	84 86	0 0	114 116	233 233	0 0	0 0
H_DRC_152	108 108	129 129	196 196	102 102	98 98	92 94	104 106	0 0	84 84	0 0	0 0	201 229	0 0	0 0
H_TAN_153	0 0	129 129	196 196	0 0	100 100	80 92	102 102	123 129	84 86	80 86	120 122	0 0	0 0	171 173
H_KEN_154	0 0	111 131	194 194	0 0	98 110	76 76	102 102	0 0	94 96	0 0	122 122	211 229	0 0	0 0
H_KEN_155	110 122	111 111	186 186	104 104	102 104	80 92	100 104	127 129	78 92	78 88	114 114	211 229	170 174	0 0
H_KEN_156	94 94	111 113	196 196	80 80	104 110	76 76	104 104	127 141	88 92	80 88	122 128	213 213	174 182	0 0
H_KEN_157	112 112	113 129	0 0	80 80	104 106	80 92	102 108	125 127	86 96	78 90	114 114	205 217	0 0	0 0
H_CPT_158	128 136	111 127	196 196	80 94	106 108	76 86	106 110	133 139	78 92	86 86	122 126	213 213	174 182	175 191
H_UNK_159	82 96	111 129	190 196	0 0	102 106	76 86	102 106	123 125	86 92	82 82	114 126	215 235	170 174	0 0
H_KEN_160	94 124	111 129	186 186	80 94	104 110	72 76	102 106	127 143	92 92	80 90	122 128	213 235	182 194	173 175
H_RSA_161	0 0	129 131	0 0	0 0	98 98	0 0	104 104	125 125	90 90	0 0	118 118	211 211	0 0	0 0
H_CAR_162	82 100	125 141	186 186	80 80	100 110	80 92	110 110	135 135	92 92	0 0	116 116	229 233	158 198	0 0

B.3.a: NOVEL 12S TO 16S MITOCHONDRIAL SEQUENCES

>Z1 KT164799.1 Panthera leo haplotype Z1 12S ribosomal RNA gene, partial sequence; tRNA-Val gene, complete sequence; and 16S ribosomal RNA gene, partial sequence; mitochondrial

CAACAGCTTAAAACTCAAAGGACTTGGCGGTGCTTTACATCCCTCTAGAGGAGCCTG
TTCTATAATCGATAAACCCCGATAAACCTCACCATCTCTTGCTAATTCAGCCTATATA
CCGCCATCTTCAGCAAACCCTAAAAAGGAAGAAAAGTAAGCACAAGTGTCTTAACA
CAAAAAAGTTAGGTCAAGGTGTAGCCTATGAGATGGGAAGCAATGGGCTACATTTT
CTACAATTAGAACACCCACGAAAATCCTTATGAAACTAAGCATTCAAGGAGGATTT
AGCAGTAAATTTGAGAATAGAGAGCTCAATTGAATCGGGCCATGAAGCACGCACAC
ACCGCCCGTCACCCTCCTCAAGTGACTAGCCCCTAAAGAAACCTATTCAAACCACTA
CATCCACAAGAGGAGACAAGTCGTAACAAGGTAAGCATACTGGAAAGTGTGCTTGG
ATGACAAGATGTAGCTTAAACTAAAGCGTCTGGCTTACACCCAGAAGATTTCATATT
AAACTGACCGTCTTGAGCCAAAGCTAGCCCAATCATCTACAAACGCAACTAACACT
AGAAAGTAAAATAAAACATTTAGTTACCCCATAAAAGTATAGGAGATAGAAATTTA
ACTTGGCGCTATAGAGAAAGTACCGCAAGGGAAGGATGAAAGAAAAAACTAAAAG
CACTATACAGCAAAGATTGCCCCTTGTACCTTTTGCATAATGAGTTAGCTAGTAACA
GCCTAACAAAGAGAACTTCAGCTAGGCCCCCCGAAACCAGACGAGCTACCCATGAA
CAATCTATTACAGGATGAACTCGTCTATGTTGCAAAATAGTGAGAAGATTTATGGGT
AGAGGTGAAAAGCCTAACGAGCCTGGTGATAGCTGGTTGCCCAGAACAGAATCTTA
GTTCAACTTTAAACTTACCTCAAAACCCTAAAATTCCAATGTAAGTTTAAATTATAG
TCTAAAAAGGTACAGCTTTTTAGAACTAGGATACAGCCTTAATTAGAGAGTAAGCAC
AAACACAAACCATAGTTGGCCTAAAAGCAGCCACCAATTAAGAAAGCGTTCAAGCT
CGACAATCAAAACATCTCAATGTCAAAAAACGTAACCAACTCCTAACCTAAAACTG
GGCTAATCTATTTAATAATAGAAGCAATAATGCTAATATGAGTAACAAGAAGCATTT
CTCCCGTGCATAAGCTTATATCAGAACGGATAACCACTGATAGTTAACAACAAGATA
GATACAACCTAACTACAAGCAAAATATCAAACTAATTGTTAACCCAACACAGGCAT
GCAATCCAGGGAAAGATTAAAAGAAGTGAAAGGAACTCGGCAAACACAAGCCCCG
CCTGTTTACCAAAAACATCACCTCTAGCATTCCCAGTATTAGAGGCACTGCCTGCCC
AGTGACATTAGTTAAACGGCCGCGGTATCCTGACCGTGCAAAGGTAGCATAATCATT
TGTTCCTTAAATAGGGACTTGTATGAATGGCCACACGAGGGCTTTACTGTCTCTTACT
TCTGATCCGTGAAATTGACCTTCCCGTGAAGAGGCGGGAATATGACAATAAGACGA
GAAGACCCTATGGAGCTTTAATTAACCGACCCAAAGAGATCTTGATAACCAACCAA
CAGGGATAACAAACCTCTACCATGGGTCGACAATTTAGGTTGGGGTGACCTCGGAG
AATAAAACAACCTCCGAGTGATTTAAATCTAGACTAACCAGTCGAAAATATTACATC
ACTTATTGATCCAAAAACTTGATCAACGGAACAAGTTACCCTAGGGATAACAGCGC
AATCCTATTTTAGAGTCCATATCGACAATAGGGTTTACGACCTCGATGTTGGATCAG
GACATCCCGATGGTGCA

>Z2 KT164800.1 Panthera leo haplotype Z2 12S ribosomal RNA gene, partial sequence; tRNA-Val gene, complete sequence; and 16S ribosomal RNA gene, partial sequence; mitochondrial

CAACAGCTTAAAACTCAAAGGACTTGGCGGTGCTTTACATCCCTCTAGAGGAGCCTG
TTCTATAATCGATAAACCCCGATAAACCTCACCATCTCTTGCTAATTCAGCCTATATA
CCGCCATCTTCAGCAAACCCTAAAAAGGAAGAAAAGTAAGCACAAGTGTCTTAACA
CAAAAAAGTTAGGTCAAGGTGTAGCCTATGAGATGGGAAGCAATGGGCTACATTTT
CTACAATTAGAACACCCACGAAAATCCTTATGAAACTAAGCATTCAAGGAGGATTT
AGCAGTAAATTTGAGAATAGAGAGCTCAATTGAATCGGGCCATGAAGCACGCACAC
ACCGCCCGTCACCCTCCTCAAGTGACTAGCCCCTAAAGAAACCTATTCAAACCACTA
CATCCACAAGAGGAGACAAGTCGTAACAAGGTAAGCATACTGGAAAGTGTGCTTGG
ATGACAAGATGTAGCTTAAACTAAAGCGTCTGGCTTACACCCAGAAGATTTCATATT
AAACTGACCGTCTTGAGCCAAAGCTAGCCCAATCATCTACAAACGCAACTAACACT
AGAAAGTAAAATAAAACATTTAGTTACCCCATAAAAGTATAGGAGATAGAAATTTA
ACTTGGCGCTATAGAGAAAGTACCGCAAGGGAAGGATGAAAGAAAAAACTAAAAG
CACTATACAGCAAAGATTGCCCCTTGTACCTTTTGCATAATGAGTTAGCTAGTAACA
GCCTAACAAAGAGAACTTCAGCTAGGCCCCCCGAAACCAGACGAGCTACCCATGAA
CAATCTATTACAGGATGAACTCGTCTATGTTGCAAAATAGTGAGAAGATTTATGGGT
AGAGGTGAAAAGCCTAACGAGCCTGGTGATAGCTGGTTGCCCAGAACAGAATCTTA
GTTCAACTTTAAACTTACCTCAAAACCCTAAAATTCCAATGTAAGTTTAAATTATAG
TCTAAAAAGGTACAGCTTTTTAGAACTAGGATACAGCCTTAATTAGAGAGTAAGCAC
AAACACAAACCATAGTTGGCCTAAAAGCAGCCACCAATTAAGAAAGCGTTCAAGCT
CGACAATCAAAACATCTCAATGTCAAAAAACGTAACCAACTCCTAACCTAAAACTG
GGCTAATCTATTTAATAATAGAAGCAATAATGCTAATATGAGTAACAAGAAGCATTT
CTCCCGTGCATAAGCTTATATCAGAACGGATAACCACTGATAGTTAACAACAAGATA
GATACAACCTAACTACAAGCAAAATATCAAACTAATTGTTAACCCAACACAGGCAT
GCAATCCAGGGAAAGATTAAAAGAAGTGAAAGGAACTCGGCAAACACAAGCCCCG
CCTGTTTACCAAAAACATCACCTCTAGCATTCCCAGTATTAGAGGCACTGCCTGCCC
AGTGACATTAGTTAAACGGCCGCGGTATCCTGACCGTGCAAAGGTAGCATAATCATT
TGTTCCTTAAATAGGGACTTGTATGAATGGCCACACGAGGGCTTTACTGTCTCTTACT
TCTGATCCGTGAAATTGACCTTCCCGTGAAGAGGCGGGAATATGACAATAAGACGA
GAAGACCCTATGGAGCTTTAATTAACCGACCCAAAGAGATCTTGATAACCAACCAA
CAGGGATAACAAACCTCTACCATGGGTCGACAATTTAGGTTGGGGTGACCTCGGAG
AATAAAACAACCTCCGAGTGATTTAAATCTAGACTAACCAGTCGAAAATATTACATC
ACTTATTGATCCAAAAACTTGATCAACGGAACAAGTTACCCTAGGGATATCAGCGCA
ATCCTATTTTAGAGTCCATATCGACAATAGGGTTTACGACCTCGATGTTGGATCAGG
ACATCCCGATGGTGCA

>Z3 KT164801.1 Panthera leo haplotype Z3 12S ribosomal RNA gene, partial sequence; tRNA-Val gene, complete sequence; and 16S ribosomal RNA gene, partial sequence; mitochondrial

CAACAGCTTAAAACTCAAAGGACTTGGCGGTGCTTTACATCCCTCTAGAGGAGCCTG
TTCTATAATCGATAAACCCCGATAAACCTCACCATCTCTTGCTAATTCAGCCTATATA
CCGCCATCTTCAGCAAACCCTAAAAAGGAAGAAAAGTAAGCACAAGTGTCTTAACA
CAAAAAAGTTAGGTCAAGGTGTAGCCTATGAGATGGGAAGCAATGGGCTACATTTT
CTACAATTAGAACACCCACGAAAATCCTTATGAAACTAAGCATTCAAGGAGGATTT
AGCAGTAAATTTGAGAATAGAGAGCTCAATTGAATCGGGCCATGAAGCACGCACAC
ACCGCCCGTCACCCTCCTCAAGTGACTAGCCCCTAAAGAAACCTATTCAAACCACTA
CATCCACAAGAGGAGACAAGTCGTAACAAGGTAAGCATACTGGAAAGTGTGCTTGG
ATGACAAGATGTAGCTTAAACTAAAGCGTCTGGCTTACACCCAGAAGATTTCATATT
AAACTGACCGTCTTGAGCCAAGGCTAGCCCAATCATCTACAAACGCAACTAACACT
AGAAAGTAAAATAAAACATTTAGTTACCCCATAAAAGTATAGGAGATAGAAATTTA
ACTTGGCGCTATAGAGAAAGTACCGCAAGGGAAGGATGAAAGAAAAAACTAAAAG
CACTATATAGCAAAGATTACCCCTTGTACCTTTTGCATAATGAGTTAGCTAGTAACA
GCCTAACAAAGAGAACTTCAGCTAGGCCCCCCGAAACCAGACGAGCTACCCATGAA
CAATCTATTACAGGATGAACTCGTCTATGTTGCAAAATAGTGAGAAGATTTATGGGT
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GTTCAACTTTAAACTTACCTCAAAAAACCCTAAAATTCCAATGTAAGTTTAAATTAT
AATCTAAAAAGGTACAGCTTTTTAGAACTAGGATACAGCCTTAATTAGAGAGTAAG
CACAAACACAAACCATAGTTGGCCTAAAAGCAGCCACCAATTAAGAAAGCGTTCAA
GCTCGACAATCAAAACATCTCAATGTCAAAAAACGTAACCAACTCCTAACCTAAAA
CTGGGCTAATCTATTTAATAATAGAAGCAATAATGCTAATATGAGTAACAAGAAGC
ATTTCTCCCGTGCATAAGCTTATATCAGAACGGATAACCACTGATAGTTAACAACAA
GATAGATACAACCTAACTACAAGCAAAATATCAAACTAATTGTTAACCCAACACAG
GCATGCAATCCAGGGAAAGATTAAAAGAAGTAAAAGGAACTCGGCAAACACAAGC
CCCGCCTGTTTACCAAAAACATCACCTCTAGCATTTCCAGTATTAGAGGCACTGCCT
GCCCAGTGACATTAGTTAAACGGCCGCGGTATCCTGACCGTGCAAAGGTAGCATAA
TCATTTGTTCCTTAAATAGGGACTTGTATGAATGGCCACACGAGGGCTTTACTGTCTC
TTACTTCTGATCCGTGAAATTGACCTTCCCGTGAAGAGGCGGGAATATGACAATAAG
ACGAGAAGACCCTATGGAGCTTTAATTAACCGACCCAAAGAGATCTTGACAATCAA
CCAACAGGGACAACAAACCTCTACCATGGGTCGACAATTTAGGTTGGGGTGACCTC
GGAGAATAAAACAACCTCCGAGTGATTTAAATCTAGACTAACCAGTCGAAAATATT
ACATCACTTATTGATCCAAAAACTTGATCAACGGAACAAGTTACCCTAGGGATAACA
GCGCAATCCTATTTTAGAGTCCATATCGACAATAGGGTTTACGACCTCGATGTTGGA
TCAGGACATCCCGATGGTGCA

>Z4 KT164802.1 Panthera leo haplotype Z4 12S ribosomal RNA gene, partial sequence; tRNA-Val gene, complete sequence; and 16S ribosomal RNA gene, partial sequence; mitochondrial

CAACAGCTTAAAACTCAAAGGACTTGGCGGTGCTTTACATCCCTCTAGAGGAGCCTG
TTCTATAATCGATAAACCCCGATAAACCTCACCATCTCTTGCTAATTCAGCCTATATA
CCGCCATCTTCAGCAAACCCTAAAAAGGAAGAAAAGTAAGCACAAGTGTCTTAACA
CAAAAAAGTTAGGTCAAGGTGTAGCCTATGAGATGGGAAGCAATGGGCTACATTTT
CTACAATTAGAACACCCACGAAAATCCTTATGAAACTAAGCATTCAAGGAGGATTT
AGCAGTAAATTTGAGAATAGAGAGCTCAATTGAATCGGGCCATGAAGCACGCACAC
ACCGCCCGTCACCCTCCTCAAGTGACTAGCCCCTAAAGAAACCTATTCAAACCGCTA
CATCCACAAGAGGAGACAAGTCGTAACAAGGTAAGCATACTGGAAAGTGTGCTTGG
ATGACAAGATGTAGCTTAAACTAAAGCGTCTGGCTTACACCCAGAAGATTTCATATT
AAACTGACCGTCTTGAGCCAAAGCTAGCCCAATCATCTACAAACGCAACTAACACT
AGAAAGTAAAATAAAACATTTAGTTACCCCATAAAAGTATAGGAGATAGAAATTTA
ACTTGGCGCTATAGAGAAAGTACCGCAAGGGAAGGATGAAAGAAAAAACTAAAAG
CACTATACAGCAAAGATTGCCCCTTGTACCTTTTGCATAATGAGTTAGCTAGTAACA
GCCTAACAAAGAGAACTTCAGCTAGGCCCCCCGAAACCAGACGAGCTACCCATGAA
CAATCTATTACAGGATGAACTCGTCTATGTTGCAAAATAGTGAGAAGATTTGTGGGT
AGAGGTGAAAAGCCTAACGAGCCTGGTGATAGCTGGTTGCCCAGAACAGAATCTTA
GTTCAACTTTAAACTTACCTCAAAACCCTAAAATTCCAATGTAAGTTTAAATTATAG
TCTAAAAAGGTACAGCTTTTTAGAACTAGGATACAGCCTTAATTAGAGAGTAAGCAC
AAACACAAACCATAGTTGGCCTAAAAGCAGCCACCAATTAAGAAAGCGTTCAAGCT
CGACAATCAAAACATCTCAATGTCAAAAAACGTAACCAACTCCTAACCTAAAACTG
GGCTAATCTATTTAATAATAGAAGCAATAATGCTAATATGAGTAACAAGAAGCATTT
CTCCCGTGCATAAGCTTATATCAGAACGGATAACCACTGATAGTTAACAACAAGATA
GATACAACCTAACTACAAGCAAAATATCAAACTAATTGTTAACCCAACACAGGCAT
GCAATCCAGGGAAAGATTAAAAGAAGTGAAAGGAACTCGGCAAACACAAGCCCCG
CCTGTTTACCAAAAACATCACCTCTAGCATTTCCAGTATTAGAGGCACTGCCTGCCC
AGTGACATTAGTTAAACGGCCGCGGTATCCTGACCGTGCAAAGGTAGCATAATCATT
TGTTCCTTAAATAGGGACTTGTATGAATGGCCACACGAGGGCTTTACTGTCTCTTACT
TCTGATCCGTGAAATTGACCTTCCCGTGAAGAGGCGGGAATATGACAATAAGACGA
GAAGACCCTATGGAGCTTTAATTAACCGACCCAAAGAGATCTTGATAATCAACCAA
CAGGGATAACAAACCTCTACCATGGGTCGACAATTTAGGTTGGGGTGACCTCGGAG
AATAAAACAACCTCCGAGTGATTTAAATCTAGACTAACCAGTCGAAAATATTACATC
ACTTATTGATCCAAAAACTTGATCAACGGAACAAGTTACCCTAGGGATAACAGCGC
AATCCTATTTTAGAGTCCATATCGACAATAGGGTTTACGACCTCGATGTTGGATCAG
GACATCCCGATGGTGCA

>Z5 KT164803.1 Panthera leo haplotype Z5 12S ribosomal RNA gene, partial sequence; tRNA-Val gene, complete sequence; and 16S ribosomal RNA gene, partial sequence; mitochondrial

CAACAGCTTAAAACTCAAAGGACTTGGCGGTGCTTTACATCCCTCTAGAGGAGCCTG
TTCTATAATCGATAAACCCCGATAAACCTCACCATCTCTTGCTAATTCAGCCTATATA
CCGCCATCTTCAGCAAACCCTAAAAAGGAAGAAAAGTAAGCACAAGTGTCTTAACA
CAAAAAAGTTAGGTCAAGGTGTAGCCTATGAGATGGGAAGCAATGGGCTACATTTT
CTACAATTAGAACACCCACGAAAATCCTTATGAAACTAAGCATTCAAGGAGGATTT
AGCAGTAAATTTGAGAATAGAGAGCTCAATTGAATCGGGCCATGAAGCACGCACAC
ACCGCCCGTCACCCTCCTCAAGTGACTAGCCCCTAAAGAAACCTATTCAAACCACTA
CATCCACAAGAGGAGACAAGTCGTAACAAGGTAAGCATACTGGAAAGTGTGCTTGG
ATGACAAGATGTAGCTTAAACTAAAGCGTCTGGCTTACACCCAGAAGATTTCATATT
AAACTGACCGTCTTGAGCCAAAGCTAGCCCAATCATCTACAAACGCAACTAACACT
AGAAAGTAAAATAAAACATTTAGTTACCCCGTAAAAGTATAGGAGATAGAAATTTA
ACTTGGCGCTATAGAGAAAGTACCGCAAGGGAAGGATGAAAGAAAAAACTAAAAG
CACTATACAGCAAAGATTGCCCCTTGTACCTTTTGCATAATGAGTTAGCTAGTAACA
GCCTAACAAAGAGAACTTCAGCTAGGCCCCCCGAAACCAGACGAGCTACCCATGAA
CAATCTATTACAGGATGAACTCGTCTATGTTGCAAAATAGTGAGAAGATTTGTGGGT
AGAGGTGAAAAGCCTAACGAGCCTGGTGATAGCTGGTTGCCCAGAACAGAATCTTA
GTTCAACTTTAAACTTACCTCAAAACCCTAAAATTCCAATGTAAGTTTAAATTATAG
TCTAAAAAGGTACAGCTTTTTAGAACTAGGATACAGCCTTAATTAGAGAGTAAGCAC
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CGACAATCAAAACATCTCAATGTCAAAAAACGTAACCAACTCCTAACCTAAAACTG
GGCTAATCTATTTAATAATAGAAGCAATAATGCTAATATGAGTAACAAGAAGCATTT
CTCCCGTGCATAAGCTTATATCAGAACGGATAACCACTGATAGTTAACAACAAGATA
GATACAACCTAACTACAAGCAAAATATCAAACTAATTGTTAACCCAACACAGGCAT
GCAATCCAGGGAAAGATTAAAAGAAGTGAAAGGAACTCGGCAAACACAAGCCCCG
CCTGTTTACCAAAAACATCACCTCTAGCATTTCCAGTATTAGAGGCACTGCCTGCCC
AGTGACATTAGTTAAACGGCCGCGGTATCCTGACCGTGCAAAGGTAGCATAATCATT
TGTTCCTTAAATAGGGACTTGTATGAATGGCCACACGAGGGCTTTACTGTCTCTTACT
TCTGATCCGTGAAATTGACCTTCCCGTGAAGAGGCGGGAATATGACAATAAGACGA
GAAGACCCTATGGAGCTTTAATTAACCGACCCAAAGAGATCTTGATAATCAACCAA
CAGGGATAACAAACCTCTACCATGGGTCGACAATTTAGGTTGGGGTGACCTCGGAG
AATAAAACAACCTCCGAGTGATTTAAATCTAGACTAACCAGTCGAAAATATTACATC
ACTTATTGATCCAAAAACTTGATCAACGGAACAAGTTACCCTAGGGATAACAGCGC
AATCCTATTTTAGAGTCCATATCGACAATAGGGTTTACGACCTCGATGTTGGATCAG
GACATCCCGATGGTGCA

>Z6 LR593884 Panthera leo haplotype Z6 12S ribosomal RNA gene, partial sequence; tRNA-Val gene, complete sequence; and 16S ribosomal RNA gene, partial sequence; mitochondrial

CAACAGCTTAAAACTCAAAGGACTTGGCGGTGCTTTACATCCCTCTAGAGGAGCCTG
TTCTATAATCGATAAACCCCGATAAACCTCACCATCTCTTGCTAATTCAGCCTATATA
CCGCCATCTTCAGCAAACCCTAAAAAGGAAGAAAAGTAAGCACAAGTGTCTTAACA
CAAAAAAGTTAGGTCAAGGTGTAGCCTATGAGATGGGAAGCAATGGGCTACATTTT
CTACAATTAGAACACCCACGAAAATCCTTATGAAACTAAGCATTCAAGGAGGATTT
AGCAGTAAATTTGAGAATAGAGAGCTCAATTGAATCGGGCCATGAAGCACGCACAC
ACCGCCCGTCACCCTCCTCAAGTGACTAGCCCCTAAAGAAACCTATTCAAACCACTA
CATCCACAAGAGGAGACAAGTCGTAACAAGGTAAGCATACTGGAAAGTGTGCTTGG
ATGACAAGATGTAGCTTAAACTAAAGCGTCTGGCTTACACCCAGAAGATTTCATATT
AAACTGACCGTCTTGAGCCAAAGCTAGCCCAATCATCTACAAACGCAACTAACACT
AGAAAGTAAAATAAAACATTTAGTTACCCCATAAAAGTATAGGAGATAGAAATTTA
ACTTGGCGCTATAGAGAAAGTACCGCAAGGGAAGGATGAAAGAAAAAACTAAAAG
CACTATACAGCAAAGATTGCCCCTTGTACCTTTTGCATAATGAGTTAGCTAGTAACA
GCCTAACAAAGAGAACTTCAGCTAGGCCCCCCGAAACCAGACGAGCTACCCATGAA
CAATCTATTACAGGATGAACTCGTCTATGTTGCAAAATAGTGAGAAGATTTATGGGT
AGAGGTGAAAAGCCTAACGAGCCTGGTGATAGCTGGTTGCCCAGAACAGAATCTTA
GTTCAACTTTAAACTTACCTCAAAACCCTAAAATTCCAATGTAAGTTTAAATTATAG
TCTAAAAAGGTACAGCTTTTTAGAACTAGGATACAGCCTTAATTAGAGAGTAAGCAC
AAACACAAACCATAGTTGGCTTAAAAGCAGCCACCAATTAAGAAAGCGTTCAAGCT
CGACAATCAAAACATCTCAATGTCAAAAAACGTAACCAACTCCTAACCTAAAACTG
GGCTAATCTATTTAATAATAGAAGCAATAATGCTAATATGAGTAACAAGAAGCATTT
CTCCCGTGCATAAGCTTATATCAGAACGGATAACCACTGATAGTTAACAACAAGATA
GATACAACCTAACTACAAGCAAAATATCAAACTAATTGTTAACCCAACACAGGCAT
GCAATCCAGGGAAAGATTAAAAGAAGTGAAAGGAACTCGGCAAACACAAGCCCCG
CCTGTTTACCAAAAACATCACCTCTAGCATTCCCAGTATTAGAGGCACTGCCTGCCC
AGTGACATTAGTTAAACGGCCGCGGTATCCTGACCGTGCAAAGGTAGCATAATCATT
TGTTCCTTAAATAGGGACTTGTATGAATGGCCACACGAGGGCTTTACTGTCTCTCAC
TTCTGATCCGTGAAATTGACCTTCCCGTGAAGAGGCGGGAATATGACAATAAGACG
AGAAGACCCTATGGAGCTTTAATTAACCGACCCAAAGAGATCTTGATAATCAACCA
ACAGGGATAACAAACCTCTACCATGGGTCGACAATTTAGGTTGGGGTGACCTCGGA
GAATAAAACAACCTCCGAGTGATTTAAATCTAGACTAACCAGTCGAAAATATTACAT
CACTTATTGATCCAAAAACTTGATCAACGGAACAAGTTACCCTAGGGATAACAGCG
CAATCCTATTTTAGAGTCCATATCGACAATAGGGTTTACGACCTCGATGTTGGATCA
GGACATCCCGATGGTGCA

B.3.b: FASTAS OF THE 280 MITOGENOMIC POLYMORPHIC SITES

>Hap_1 Panthera leo Mitogenomic Polymorphic Sites GIR_050
ACCTTCCGTCCCTTACTGGTAGCCGTGCTTTATCCTAATGAGCGCGCCTCACTGCACC
ATCAACACTGCCTGGTCATTATTAAAACTATCGAAGTTGCGTTTCATCACTGTCTAAT
TAGATCTAGGTTACTTAACATAAGGCGATTTTCTTCCATATTACCTCTCATCTCGTCG
GTCACCCGGTCTTGCTTTAACCTCTTGCTACCGCCTAACGCGTAGTTACTTCTAACGT
CTCTGTGTGGGCATTGCGCTCTACTTCCCCAATATTACTTTAATCGTA

>Hap_2 Panthera leo Mitogenomic Polymorphic Sites GIR_054
ACTTTCCGTCCCTTACTGGTAGCCGTACTTTATCCTAACAAACGCGCCTCACTGCGTC
ATCAACACTGCCTGGCTATTATTAAAACTATCGAAGTTGCGTTTCATCACTGTCTAAT
TAGATCTAGGTTACTTAACATAAGGCGATTTTCTTCCGTATTACCTCTCATCTCGCCG
GTCACCCGGTCTTGCTTTAACCTCTTGCTACCGCCTAACGCGCAGTCACTTCTAACGT
CTCTGTGTGGGCATTGCGCTCTACTTCCCCAATATTACTTTAATCGTA

>Hap_3 Panthera leo Mitogenomic Polymorphic Sites GIR_051
ACTTTCCGTCCCTTACTGGTAGCCGTACTTTATCCTAACAAGCGCGCCTCACTGCGTC
ATCAACACTGCCTGGCCATTATTAAAACTATCGAAGTTGCGTTTCATCACTGTCTAAT
TAGATCTAGGTTACTTAACATAAGGCGATTTTCTTCCGTATTACCTCTCATCTCGCCG
GTCACCCGGTCTTGCTTTAACCTCTTGCTACCGCCTAACGCGCAGTCACTTCTAACGT
CTCTGTGTGGGCATTGCGCTCTACTTCCCCAATATTACTTTAATCGTA

>Hap_4 Panthera leo Mitogenomic Polymorphic Sites GIR_120
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ATCAACACTGCCTGGCCATTATTAAAACTATCGAAGTTGCGTTTCATCACTGTCTAAT
TAGATCTAGGTTACTTAACATAAGGCGATTTTCTTCCGTATTACCTCTCATCTCGCCG
GTCACCCGGTCTTGCTTTAACCTCTTGCTACCGCCTAACGCGCAGTCACTTCTAACGT
CTCTGTGTGGGCATTGCGCTCTACTTCCCCAATATTACTTTAATCGTA

>Hap_5 Panthera leo Mitogenomic Polymorphic Sites GIR_KC
ACCTTCCGTCCCTTACTGGTAGCCGTACTTTATCCTAACAAACGCGCCTCACTGCGTC
ATCAACACTGCCTGGCCATTATTAAAACTATCGAAGTTGCGTTTCATCACTGTCTAAT
TAGATCTAGGTTACTTAACATAAGGCGATTTTCTTCCGTATTACCTCTCATCTCGCCG
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>Hap_6 Panthera leo Mitogenomic Polymorphic Sites GIR_KP501
ACCTTCCGTCCCTTACTGGTAGCCGTACTTTATCCTAACAAACGCGCCTCACTGCGTC
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TAGATCTAGGTTACTTAACATAAGGCGATTTTCTTCCGTATTACCTCTCATCTCGCCG
GTCACCCGGTCTTGCTTTAACCTCTTGCTACCGCCTAACGCGCAGTCACTTCTAACGT
CTCTGTGTGGGCATTGCGCTCTACTTCCCCGATATTACTTTAATCGTA

>Hap_7 Panthera leo Mitogenomic Polymorphic Sites DRC_033
ACTTTCTATCCCTCATTGGTAGCCATGCTTTACTCCAATGAGCGCGTCCTGCTATACC
ATCAGAACTACCTGATTATTATTAAAACTACCGAAGTTGTAACCCACCGCCATCTAA
TTAGATCTAGGTCGTCTGACACAAGGTGACTCTCTTCTGTACTACCTCTCATCTCGTC
AGCCGCCCAGTCCTACTCTAGCCTCTTACTACCATCTAACATGCAGTTACCTTTAATG
TCTCCACGTGGGCTTTGCATCTAACTCCCCCAATATCACCCCAACTGTA

>Hap_8 Panthera leo Mitogenomic Polymorphic Sites BEN_KP497
GCCTTCCGTCCCTCATTGGCAGCCATACTTTACCCTAACAAACGCGTCCCGCTGCGT
CATCAGAACTGCCTGGCCATTGTTAAAACTACCGAAGTTGTAATCCATCACCGCCTA
ATTAGATCTAGGTTACTTAACATAGGGCGATTCTCTTCTGTATTACCTCTCATCTCGC
CAGCCACCCGGTCTTGCTTTAGCCTCTTGCTACCACCTAACGTGTAGTCACTTCTAAT
GTCTCCGTGTGGGCATTGCATTCTACTTCCCCGATATTATCCCGATTGTA

>Hap_9 Panthera leo Mitogenomic Polymorphic Sites DRC_038
GCCTTCCGTCCCTCACCGGTAGCCATGCTTTACCCTGATGAGCGCGTTCTGCTGTACC
GTTAGAACTGCCTGACCATTATTAGAACTACCGAAGTTGTAACCCATTATCACCTAA
TTAGATCTAGGTTGCTTGACACAAGGTGACTTTCTTCTGTATTACCTCTCATCTCGTC
AGCCATCCAGTTTTACTTTAGCCTCTTGCTACCATCCAACGTATAGTCACCTCTAATG
TCCCCGTGTGGGCTTTGCATCCTACTTCCCCAATATTACCCCAATTGTA

>Hap_10 Panthera leo Mitogenomic Polymorphic Sites SOM_113
GCCTTCTATCCCTCACTGATAGCCATGCTTTACCCCAATGAGCGCGTCCCGCTGTGTC
ATTAGAACTACCTGACTATTGTTAAAACCACTGAAGTTGTAACCCATCACCATCTAA
TTAGATCTAGGTTATCTGACACAAGGCGACTCTCTCCTGTACTACCCCTCATCTCGTC
AGCCGCCCGGTCCTACTTTGGCCTCTTGCTACCACCTAACATGCAGTTACCTCTAATG
TCCCCGTGTGGGCTTTGCATTCTACTTCCCCAATATTACCCCAATTGTA

>Hap_11 Panthera leo Mitogenomic Polymorphic Sites DRC_035
GCCTTCTATCCCTCACTGGTAGCCATACTTTACCCTGATGAGCGCGTTCCACTGTACC
GTTAGAACTACCTGACCATTATTAAAACTACCGAAGTTGTAACCCATCGCCACCTAA
TTAGATCTAGGTTATCTGACACAAGGCGACTCTCTTCTGTATTACCTCTCATCTCGCC
AGCCGCCCGGTCCTACTTTGGCCTCTTGCTACCACCTAACATGCAGTCACCTCTAAT
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>Hap_12 Panthera leo Mitogenomic Polymorphic Sites DRC_040
GCCTTCTATCCCTCACTGGTAGCCATACTTTACCCCGACAAGCGCGTCCTGCTGTGTC
GTTAGAACTACCTGACCATTATTAAAACTACCGAAGTTGTAACCCATCGCCACCTAA
TTAGATCTAGGTTATCTGACACAAGGCGACTTTCTTCTGTATTACCTCTCATCTCGCC
AGCCGCCCGGTCCTACTTTAGCCTCTTGCTACCACCTAACATGCAGTCACCTCTAAT
GTCCCCGTGTGGGCTTTGCATTCTACTTCCCCGACGTTACCCCAATTGTA

>Hap_13 Panthera leo Mitogenomic Polymorphic Sites DRC_031
GCCTTCTATCCCTCACTGGTAGCCATACTTTACCCCGACAAGCGCGTCCTGCTGTGTC
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TTAGATCTAGGTTATCTGACACAAGGCGACTTTCTTCTGTATTACCTCTCATCTCGCC
AGCCGCCCGGTCCTACTTTAGCCTCTTGCTACCACCTAACATGCAGTCACCTCTAAT
GTCCCCGTGTGGGCTTTGCATTCTACTTCCCCAATATTACCCCAATTGTA

>Hap_14 Panthera leo Mitogenomic Polymorphic Sites CAR_067, DRC_043
GCCTTCTATCCCTCACTGGTAGCCATACTTTACCCCGACAAACGCGTCCTGCTGTGTC
GTTAGAACTACCTGACCATTATTAAAACTACCGAAGTTGTAACCCATCGCCACCTAA
TTAGATCTAGGTTATCTGACACAAGGCGACTTTCTTCTGTATTACCTCTCATCTCGCC
AGCCGCCCGGTCCTACTTTAGCCTCTTGCTACCACCTAACATGCAGTCACCTCTAAT
GTCCCCGTGTGGGCTTTGCATTCTACTTCCCCAATATTACCCCAATTGTA

>Hap_15 Panthera leo Mitogenomic Polymorphic Sites DRC_036, DRC_042
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GTTAGAACTACCTGACCATTATTAAAACTACCGAAGTTGTAACCCATCGCCACCTAA
TTAGATCTAGGTTATCTGACACAAGGCGACTTTCTTCTGTATTACCTCTCATCTCGCC
AGCCGCCCGGTCCTACTTTAGCCTCTTGCTACCACCTAACATGCAGTCACCTCTAAT
GTCCCCGTGTGGGCTTTGCATTCTACTTCCCCAATATTACCCCAATTGTA

>Hap_16 Panthera leo Mitogenomic Polymorphic Sites DRC_039
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ATTAGAACTACCTGACCATTACTAAAACTACCGAAGTTGTAACCCACCGCCATCTAA
TTAGATCTAGGTTATCTGACACAAGGCGACTTTCTTCTGTATTACCTCTCATCTCGCC
AGCCGCCCGGTCCTACTTCAGCCTCTTGCTACCACCTAACATGCAGTCACCTCTAAT
GTCCCCGTGTGGGCTTTGCATTCTACTTCCCCAATATCACCCCAATTGTA

>Hap_17 Panthera leo Mitogenomic Polymorphic Sites DRC_KP506
GCCTTCTATCCCTCACTGGTAGCCATACTTTACCCCAACAAACGCGTCCTGCTGTGTC
ATTAGAACTACCTGACCATTACTAAAACTACCGAAGTTGTAACCCACCGCCATCTAA
TTAGATCTAGGTTATCTGACACAAGGCGACTTTCTTCTGTATTACCTCTCATCTCGCC
AGCCGCCCGGTCCTACTTCAGCCTCTTGCTACCACCTAACATGCAGTCACCTCTAAT
GTCCCCGTGTGGGCTTTGCATTCTACTTCCCCGATATCACCCCAATTGTA

>Hap_18 Panthera leo Mitogenomic Polymorphic Sites KEN_057
GCCTTCTATCCCTCACTGATAGCCATACTTTACCCCAATGAGCGCGTCCTGCTGTGTC
ATTAGAACTACCCGACCATTATTGAAACTACCGAAGTTGTAACCCATCGCCATCTAA
TTAGATCTAGGTTATCTGACACAAGGCGACTTTCTTCTGTATTACCTCTCATCTCGCC
AGCCGCCCGGTCCTACTTTAGCCTCTTGCTACCACCTAACATGCAGTCACCTCTAAT
GTCCCCGTGTGGGCTTTGCATTCTACTTCTCCAATATCACCCCAATTGTA

>Hap_19 Panthera leo Mitogenomic Polymorphic Sites PLE-KP262
GCCTTCTATCCCTCACTGATAGCCATACTTTACCCCAACAAACGCGTCCTGCTGTGTC
ATTAGAACTACCCGACCATTATTGAAACTACCGAAGTTGTAACCCATCGCCATCTAA
TTAGATCTAGGTTATCTGACACAAGGCGACTTTCTTCTGTATTACCTCTCATCTCGCC
AGCCGCCCGGTCCTACTTTAGCCTCTTGCTACCACCTAACATGCAGTCACCTCTAAT
GTCCCCGTGTGGGCTTTGCATTCTACTTCTCCGATATCACCCCAATTGTA

>Hap_20 Panthera leo Mitogenomic Polymorphic Sites DRC_KP494
GCCTTCTATCCCTCACTGATAGCCATACTTTACCCCAACAAACGCGTCCTGCTGTGTC
ATTAGAACTACCCGACCATTATTAAAACTACTGAAGTTGTAACCCATCGCCATCTAA
TTAGATCTAGGTTATCTGACACAAGGCGACTTTCTTCTGTATTACCTCTCATCTCGCC
AGCCGCCCGGTCCTACTTTAGCCTCTTGCTACCACCTAACATGCAGTCACCTCTAAT
GTCCCCGTGTGGGCTTTGCATTCTACTTCCCCGATATTACCCCAATTGTA

>Hap_21 Panthera leo Mitogenomic Polymorphic Sites CAM_KP502
GCCTTCTATCCCTCACTAGTAGCCATACTTTACCCCAACAAACGTGTCCTGCTGTGTC
ATTAGAACTACCTGACCATTATTAAAACTACCGAAGTTGTAACCCATCGCCATCTAA
TTAGATCTAGGTTACCTGACACAAGGCGACTTTCTTCTGTATTACCTCTCATCCCGCC
AGCCGCCCGGTCCTACTTTAGCCTCTTGCTACCACCTAACATGCAGTCACCTCTAAT
GTCCCCGTGTGGGCTTTGCATTCTACTTCCCCGATATTACCCCAATTGTA

>Hap_22 Panthera leo Mitogenomic Polymorphic Sites CAM_KP493
GCCTTCTATCCCTCACTGGTAGCCATACTTTACCCCAACAAACGCGTCCTGCTGTGTC
ATTAGAACTACCTGACCATTATTAAAACTACCGAAGTTGTAACCCATCGCCATCTAA
TTAGATCTAGGTTACCTGACACAAGGCGACTTTCTTCTGTATTACCTCTCATCTCGCC
AGCCGCCCGGTCCTACTTTAGCCTCTTGCTACCACCTAACATGCAGTCACCTCTAAT
GTCCCCGTGTGGGCTTTGCATTCTACTTCCCCGATATTACCCCAATTGTA

>Hap_23 Panthera leo Mitogenomic Polymorphic Sites ETH_KP495
GCCTTCTATCCCTCACTGGTAGCCATACTTTACCCCAACAAACGCGTCCTGCTGTGTC
GTTAGAACTACCTGACCATTATTAAAACTACCGAAGTTGTAACCCATCGCCATCTAA
TTAGATCTAGGTTATCTGACACAAGGCGACTTTCTTCTGTATTACCTCTCATCTCGCC
AGCCGCCCGGTCCTACTTTAGCCTCTTGCTACCACCTAACATGCAGTCACCTCTAAT
GTCCCCGTGTGGGCTTTGCATTCTACTTCCCCGATATTACCCCAATTGTA

>Hap_24 Panthera leo Mitogenomic Polymorphic Sites KEN_046
ACTCTCCGCCCCTCATTAGTAGCTATGTTTTACTCTAATGAGCACGTTCCACCGTACC
ACCAGAGTTGTCTGGTTATCGTTAAAACCACCGAGGTTGTAATCCGTTATCGCCCGG
CCGAGTCTGGGTCGCTCAACATGAAGCGATCCTCCCCTGTGCCATCTTCCGTTCCGT
CAGCCATTTAGTTTTGCTCTAGCTCTTTATTATTATTTAGCGTGTGGTCGCTTCTAATG
TTTCCACGTGAGCATTGCATCCAACTTTCCCAACGTTACCCCAATTGTA

>Hap_25 Panthera leo Mitogenomic Polymorphic Sites KEN_092
ACTCTCCGTCCCTCATTGGTAGCTATGTTTTACTCTAGTAAGCACGTCCCACTATGTT
ACCAGAGTTGTCTGGTTATTGTTAAAACCACCGAGGTTGTAATCCGTTATCGCCCGA
CCGAGCCTGGGTTGCTCAACATGAAGCGATCCCCCCCTGTGCCATCCTCCATTCCGC
CAACCATTTAGTTTTGCTCTAGCTCTTTATTGTTATTTGGCGTGTGGTCGCTCCTAAT
GTTTCCACGTGAGCATTGCATCCAGCTTTCCCAACGTTACCCCAACTGTA

>Hap_26 Panthera leo Mitogenomic Polymorphic Sites KEN_096
ACTTTCCGCCCCTCATTAGTAGCTATATTTTACTCTAGTAAGCATGTCCCACCGTGTT
ACCAGAGTTGTCTAGTTATCGTTAAAACCACCGAGGTTGTAATCCGTTATCGCCCGG
CCGAGCCTGGGTTGCTCAACATGAAGCGATCCCCCCCTGTGCCATCTTCCGTTCCGT
CAACCATTTAGTTTTGCTCTAGCTCTTTATTGTTATTTGGCGTGTGGTCGCTCCTAAT
GTTTCCACGTGAGCATTGCATCCAGCTTTCCCAATACCACCCCAATTACA

>Hap_27 Panthera leo Mitogenomic Polymorphic Sites KEN_091
ACTCTCCGCCCCTCATTAGTAGCTATATTTTACTCTAGTAAGCATGTCCCACCGTGTT
ACCAGAGTTGTCTAGTTATCGTTAAAACCACCGAGGTTGTAATCCGTTATCGCCCGG
CCGAGCCTGGGTTGCTCAACATGAAGCGATCCCCCCCTGTGCCATCTTCCGTTCCGC
CAACCATTTAGTTTTGCTCTAGCTCTTTATTGTTATTTAGCGTGTGGTCGCTCCTAAT
GTTTCCACGTGAGCATTGCATCCAGCTTTCCCAACGTTACCCCAATTATA

>Hap_28 Panthera leo Mitogenomic Polymorphic Sites KEN_095
ACTCTCCGCCCCTCATTAGTAGCTATATTTTACTCTAGTAAGCGCGTCCCACCGTGTT
ACCAGAGTTGTCTAGTTATCGTTAAAACCACCGAGGTTGTAATCCGTTATCGCCCGG
CCGAGCCTGGGTTGCTCAACATGAAGCGATCCCCCCCTGTGCCATCTTCCGTTCCGC
CAACCATTTAGTTTTGCTCTAGCTCTTTATTGTTATTTGGCGTGTGGTCGCTCCTAAT
GTTTCCACGTGAGCATTGCATCCAGCTTTCCCAATACCACCCCAATTATA

>Hap_29 Panthera leo Mitogenomic Polymorphic Sites KEN_044
ACTCTCCGCCCCTCATTAGTAGCTATATTTTACTCTAGTAAGCATGTCCCACCGTGTT
ACCAGAGTTGTCTAGTTATCGTTAAAACCACCGAGGTTGTAATCCGTTATCGCCCGG
CCGAGCCTGGGTTGCTCAACATGAAGCGATCCCCCCCTGTGCCATCTTCCGTTCCGC
CAACCATTTAGTTTTGCTCTAGCTCTTTATTGTTATTTGGCGTGTGGTCGCTCCTAAT
GTTTCCACGTGAGCATTGCATCCAGCTTTCCCAATACCACCCCAATTATA

>Hap_30 Panthera leo Mitogenomic Polymorphic Sites KEN_045
ACTCTCCGCCCCTCATTAGTAGCTATATTTTACTCTAGTAAGCATGTCCCACCGTGTT
ACCAGAGTTGTCTAGTTATCGTTAAAACCACCGAGGTTGTAATCCGTTATCGCCCGG
CCGAGCCTGGGTTGCTCAACATGAAGCGATCCCCCCCTGTGCCATCTTCCGTTCCGC
CAACCATTTAGTTTTGCTCTAGCTCTTTATTGTTATTTAGCGTGTGGTCGCTCCTAAT
GTTTCCACGTGAGCATTGCATCCAGCTTTCCCAATACCACCCCAATTATA

>Hap_31 Panthera leo Mitogenomic Polymorphic Sites KEN_094
ACTCTCCGCCTCTCATTAGTAGCTATATTTTACTCTAATAAGCATGTCCCACCGTGTT
ACCAGAGTTGTCTAGTTATCGTTAAAACCACCGGGGTTGTAATCCGTTATCGCCCGG
CCGAGCCTGGGTTGCTCAACATGAAGCGATCCCCCCCTGTGCCATCTTCCGTCCCGT
CAACCATTTAGTTTTGCTCTAGCTCTTTATTGTTATTTGGCGTGTGGTCACTCCTAAT
GTCTCCACGTGAACATTGCATCCAGCTTTCCCAATACCACCCCAATTACA

>Hap_32 Panthera leo Mitogenomic Polymorphic Sites KEN_016
ACTCTCCGCCCCTCATTAGTAGCTATATTTTACTCTAGTAAGCATGTCCCACCGTGTT
ACCAGAGTTGTCTAGTTATCGTTAAAACCGCCGAGGTTGTAATCCGTTATCGCCCGG
CCGAGCCTGGGTTGCTCAACATGAAGCGATCCCCCCCTGTGCCATCTTCCGTCCCGC
CAACCATTTAGTTTTGCTCTAGCTCTTTATTGTTATTTGGCGTGTGGTCACTCCTAAT
GTCTCCACGTGAGCATTGCATCCAGCTTTCCCAATACCACCCCAATTGTA

>Hap_33 Panthera leo Mitogenomic Polymorphic Sites KEN_155, SOM_KP499
ACTCTCCGCCCCTCATTAGTAGCTATATTTTACTCTAGTAAGCATGTCCCACCGTGTT
ACCAGAGTTGTCTAGTTATCGTTAAAACCGCCGAGGTTGTAATCCGTTATCGCCCGG
CCGAGCCTGGGTTGCTCAACATGAAGCGATCCCCCCCTGTGCCATCTTCCGTCCCGC
CAACCATTTAGTTTTGCTCTAGCTCTTTATTGTTATTTGGCGTGTGGTCACTCCTAAT
GTCTCCACGTGAGCATTGCATCCAGCTTTCCCAATACCACCCCAATTACA

>Hap_34 Panthera leo Mitogenomic Polymorphic Sites KEN_157
ACTCTCCGCCCCTCATTAGTAGCTATATTTTACTCTAGTAAGCATGTCCCACCGTGTT
ACCAGAGTTGTCTAGTTATCGTTAAAACCGCCGAGGTTGTAATCCGTTATCGCCCGG
CCGAGCCTGGGTTGCTCAACATGAAGCGATCCCCCCCTGTGCCATCTTCCGTCCCGC
CAACCATTTAGTTTTGCTCTAGCTCTTTATTGTTATTTAGCGTGTGGTCACTCCTAAT
GTCTCCACGTGAGCATTGCATCCAGCTTTCCCAATACCACCCCAATTACA

>Hap_35 Panthera leo Mitogenomic Polymorphic Sites BOT_072
ACTTTCCGTTCTTCGTTGGTAGCCATGCTTTACTCTAATGAGCGCGTCCCACTGTGTC
ATCAGAACTGTCTGGTTATTGTTAAAACCACCGAGGTTGTAATCCGTTATCGCCTGA
TTAGGTCTGGGTTGCTTAACATAAGGCGATTCTCCCCTATACTACCCTCCATCTCGTC
AGCCATTCAGTTTTGCTCTAGCTTCTTACTACCATCCAGCGTGTAGTCACTTCTAATG
TCTCCACGTAAGCATTGCATCCAACTTCCCCAACGTTACCCCAACTGTA

>Hap_36 Panthera leo Mitogenomic Polymorphic Sites KEN_156
ACTTTTCGTCCCTCATCGGTAGCCATGCTCTACTCTAATGAGCGCGTTCCACTATACC
ATCAGAACTGTCTGGTTACTGTTAAAGTCACCGAGGTTGTAATCCGTTATCGCCTGA
TTAGGTCTAGGTCGCTTAATATAAGGTGATTCTCCCCTGTACTACCCTCCATCTCGTC
AGCTATTTAGTTTTGCTCTGGTTTCTTACTACCATCCAGCGTATAGTCACTTCTAATG
TCTCCACATGAGCATTGCATCTAATTCCCCCAACGTTACCCCAACTGTG

>Hap_37 Panthera leo Mitogenomic Polymorphic Sites TAN_TLS
ACTTTTTGTCCCTCATCGGTAGCCATGCTCTACTCTAATGAGCGCGTTCCACTATACC
ATCAGAACTGTCTGGTTACTGTTAAAGTCACCGAGGTTGTAATCCGTTATCGCCTGA
TTAGGTCTAGGTCGCTTAATATAAGGTGATTCTCCCCTGTACTACCCTCCATCTCGTC
AGCTATTTAGTTTTGCTCTGGTTTCTTACTACCATCCAGCGTATAGTCACTTCTAATG
TCTCCACATGAGCATTGCATCTAATTCCCCCAACGTTGCCCCAACTGTG

>Hap_38 Panthera leo Mitogenomic Polymorphic Sites ZAM_KP503
ACTTTCCGTCCCTCATCGGTAGCCATGCTCTACTCTAATGAGCGCGTCCCACTATACC
ATCAGAACTGTCTGGTTACTGTTAAAGTCACCGAGGTTGTAATCCGTTATCGCCTGA
TTAGGTCTAGGTCGCTTAATATAAGGTGATTCTCCCCTGTACTACCCTCCATCTCGTC
AGCTATTTAGTTTTGCTCTGGTTTCTTACTACCATCCAGCGTATAGTCACTTCTAATG
TCTCCACATGAGCATTGCATCTAATTCCCCCAACGTTGCCCCAACTGTG

>Hap_39 Panthera leo Mitogenomic Polymorphic Sites ZAM_KP505, ZAM_T256, ZAM_T429
ACTTTCCGTCCCTCATCGGTGGCCATGCTTTACTCTAATGAGCGCGTTCCACTATACC
ATCAGAACTGTCTGGTTACTGTTAAAATCACCAAGGTTGTAATCCGCTATCGCCTGA
TTAGGTCTAGGTCGCTTAATATAAGGTGATTCTCCCCTATACTACCCTCCATCTCGTC
AGCCATTTAGTTTTGCTCTGGCTTCTTACTACCATCCAGCGTATAGTTACTTTTAATG
TCTCCACGTGAGCATTGCATCTAACTCCCCCAACGTTACCCCAACTGTA

>Hap_40 Panthera leo Mitogenomic Polymorphic Sites COG_012
ACTTTCCGTCCCTCATCGGTAGTCATGCTTTACTCTAATGAGCGCGTTCCATTATACC
ATCAGAACTGTCTGGTTACTGTCAAAATCACCGAGCTTGTAATCTGTTATCGCCTGA
TTAAGTCTAGGTCGCTTAATATAAGGTGGTTCTCCCCTGTACTGCCCTCCATCTTATC
AGCCATTTAGTTTTGCTCTGGCTTCTTACTACCATCCAGTGTATAGTTACTTCTAATG
TCTCCACGTGAGCATTGCATCTAATTCCCCCAACGTTGCCCCAGCTGTA

>Hap_41 Panthera leo Mitogenomic Polymorphic Sites COG_013
ACTTTCCGTCCCTCATCGGTAGTCATGCTTTACTCTAATGAGCGCGTTCCATTATACC
ATCAGAACTGTCTGGTTACTGTTAAAATCACCGAGCTTGTAATCTGTTATCGCCTGAT
TAAGTCTAGGTCGCTTAATATAAGGTGGTTCTCCCCTGTACTGCCCTCCATCTTATCA
GCCATTTAGTTTTGCTCTGGCTTCTTACTACCATCCAGTGTATAGTCACTTCTAATGT
CTCCACGTGAGCATTGCATCTAATTCCCCCAACGTTGCCCCAGCTGTA

>Hap_42 Panthera leo Mitogenomic Polymorphic Sites MWI_011
ACTTTCCGTCCCTCATCGGTAGTCATGCTTTACTCTAATGAGCGCGTTCCATTATACC
ATCAGAACTGTCTGGTTACTGTCAAAATCACCGAGCTTGTAATCTGTTATCGCCTGA
TTAAGTCTAGGTCGCTTAATATAAGGTGGTTCTCCCCTGTACTGCCCTCCATCTTATC
AGCCATTTAGTTTTGCTCTGGCTTCTTACTACCATCCAGTGTATAGTCACTTCTAATG
TCTCCACGTGAGCATTGCATCTAATTCCCCCAACGTTACCCCAGCTGTA

>Hap_43 Panthera leo Mitogenomic Polymorphic Sites KEN_026
ACTTTCCGTCCCTCATCGGTAGTCATGCTTTGCTCTAATGAGCGCGTTCCATTATACC
ATCAGAACTGTCTGGTTACTGTTAAAATCACCGAGCTTGTAATCTGTTATCGCCTGAT
TAAGTCTAGGTCGCTTAATATAAGGTGGTTCTCCCCTGTACTACCCTCCATCTTATCA
GCCATTTAGTTTCGCTCTGGCTTCTCACTACCATCCAGTGTATAGTCACTTCTAATGT
CTCCACGTGAGCATTACATCTAATTCCCCCAACGTTACCCCAACTGTA

>Hap_44 Panthera leo Mitogenomic Polymorphic Sites TAN_105
ACTTTCCGTCCCTCATCGGTAGTCATGCTTTACTCTAATGAGCGCGTTCCATTATACC
ATCAGAACTGTCTGGTTGCTGTTAAAATCACCAAGCTTGTAATCTGTTATCGCCTGAT
TAAGTCTAGGTCGCTTAATATAAGGTGGTTCTCCCCTATACTACCCTCCATCTTATCA
GCCATTTAGTTTTGCTCTGGCTTCTTACTACCATCCAGTGTATAGTCACTTCTAATGT
CTCCACGTGAGCATTGCATCTAACTCCCCCAACGTTGCCCCAACTGTA

>Hap_45 Panthera leo Mitogenomic Polymorphic Sites TAN_090
ACTTTCCGTCCCTCATCGGTAGTCATGCTTTACTCTAATGAGCGCGTTCCATTATACC
ATCAGAACTGTCTGGTTACTGTTAAAATCACCAAGCTTGTAATCTGTTATCGCCTGAT
TAAGTCTAGGTCGCTTAATATAAGGTGATTCTCCCCTGTACTACCCTCCATCTTATCA
GCCATTTAGTTTTGCTCTGGCTTCTTACTACCATCCAGTGTATAGTCACTTCTAATGT
CTCCACGTGAGCATTGCATCTAATTCCCCCAACGTTACCCCAACTGTA

>Hap_46 Panthera leo Mitogenomic Polymorphic Sites ETH_104
ACTTTCCGTCCCTCATCGGTAGTCATGCTTTACTCTAATGAGCGCGTTCCATTATACC
ATCGGAACTGTCTGGTTACTGTTAAAATCACCGAGCTTGTAATCTGTTATCGCCTGAT
TAAGTCTAGGTCGCTTAATATAAGGTGGTTCTCCCCTGTACTACCCTCCATCTTATCA
GCCATTTAGTTTTGCTCTGGCTTCTTACTACCATCCAGTGTATAGTCACTTCTAATGT
CTCCACGTGAGCATTGCATCTAATTCCCCCAACGTTACCCCAACTGTA

>Hap_47 Panthera leo Mitogenomic Polymorphic Sites KEN_028, KEN_029
ACTTTCCGTCCCTCATCGGTAGTCATGCTTTACTCTAATGAGCGCGTTCCATTATACC
ATCGGAACTGTCTGGTTACTGTTAAAATCACCGAGCTTGTAATCTGTTATCGCCTGAT
TAAGTCTAGGTCGCTTAATATAAGGTGGTTCTCCCCTGTACTACCCTCCATCTTATCA
GCCATTTAGTTTTGCTCTGGCTTCTTACTACCATCCAGTGTATAGTCACTTCTAATGT
CTCCACGTGAGCATTGCATCTAATTCCCCCAACGTTGCCCCAACTGTA

>Hap_48 Panthera leo Mitogenomic Polymorphic Sites TAN_145
ACTTTCCGTCCCTCATCGGTAGTCATGCTTTACTCTAATGAGCGCGTTCCATTATACC
ATCAGAACTGTCTGGTTACTGTTAAAATCACCGAGCTTGTAATCTGTTATCGCCTGAT
TAAGTCTAGGTCGCTTAATATAAGGTGGTTCTCCCCTGTACTACTCTCCATCTTATCA
GCCATTTAGTTTTGCTCTGGCTTCTTACTACCATCCAGTGTATAGTCACTTCTAATGT
CTCCACGTGAGCATTGCATCTAATTCCCCCAACGTTGCCCCAACTGTA

>Hap_49 Panthera leo Mitogenomic Polymorphic Sites NAM_014
ACTTTCCGTCCCTCATCGGTAGTCATGCTTTACTCTAATGAGCGCGTTCCATTATACC
ATCAGAACTGTCTGGTTACTGTTAAAATCACCGAGCTTGTAATCTGTTATCGCCTGAT
TAAGTCTAGGTCGCTTAATATAAGGTGGTTCTCCCCTGTACTACCCTCCATCTTATCA
GCCATTTAGTTTTGCTCTGGCTTCTTACTACCATCCAGTGTATAGTCACTTCTAATGT
CTCCACGTGAGCATTGCATCTAATTCCCCCAACGTTACCCCAACTGTA

>Hap_50 Panthera leo Mitogenomic Polymorphic Sites BOT_127, RSA_063, RSA_064, RSA_065
ACTTTCCGTCCCTCATCGGTAGTCATGCTTTACTCTAATGAGCGCGTTCCATTATACC
ATCAGAACTGTCTGGTTACTGTTAAAATCACCGAGCTTGTAATCTGTTATCGCCTGAT
TAAGTCTAGGTCGCTTAATATAAGGTGGTTCTCCCCTGTACTACCCTCCATCTTATCA
GCCATTTAGTTTTGCTCTGGCTTCTTACTACCATCCAGTGTATAGTCACTTCTAATGT
CTCCACGTGAGCATTGCATCTAATTCCCCCAACGTTGCCCCAACTGTA

>Hap_51 Panthera leo Mitogenomic Polymorphic Sites DRC_034
ACTTTCCGTCCCTCATCGGTAGTCATGCTTTACTCTAATGAGCGCGTTCCATTATACC
ATCAGAACTGTCTGGTTGCTGTTAAAATCACCGAGCTTGTAATCTGTTATCGCCTGAT
TAAGTCTAGGTCGCTTAATATAAGGTGGTTCTCCCCTGTACTACCCTCCATCTTATCA
GCCATTTAGTTTTGCTCTGGCTTCTTACTACCATCCAGTGTATAGTCACTTCTAATGT
CTCCACGTGAGCATTGCATCTAATTCCCCCAACGTTGCCCCAACTGTA

>Hap_52 Panthera leo Mitogenomic Polymorphic Sites TAN_140
ACTTTCCGTCCCTCATCGGTAGTCATGCTTTACTCTAATGAGCGCGTTCCATTATACC
ATCAGAACTGTCTGGTTACTGTTAAAATCACCGAGGTTGTAATCCGCTATCGCCTGA
TTAAGTCTAGGTCGCTTAATATAAGGTGGTTCTCCCCTGTACTACTCTCCATCTTGTC
AGCCATTTAGTTTTGCTCTGGCTTCTTACTACCATCCAGTGTATAGTCACTTTTAATG
TCTCCACGTGAGCATTGCATCTAATTCCCCCAACGTTACCCCAACTGTA

>Hap_53 Panthera leo Mitogenomic Polymorphic Sites TAN_108
ACTTTCCGTCCCTCATCGGTGGTCATGCTTTACTCTAATGAGCGCGTTCCATTATACC
ATCAGAACTGTCTGGTTGCTGTTAAAATCACCGAGCTTGTAATCTGTTATCGCCTGAT
TAAGTCTAGGTCGCTTAATATAAGGTGGTTCTCCCCTATACTACTCTCCATCTTATCA
GCCATTTAGTTTTGCTCTGGCTTCTTACTACCATCCAGCGTATAGTCACTTCTAATGT
CTCCACGTGAGCATTGCATCTAATTCCCCCAACGTTACCCCAACTGTA

>Hap_54 Panthera leo Mitogenomic Polymorphic Sites TAN_085
ACTTTCCGTCCCTCATCGGTGGCCATGCTTTACTCTAATGAGCGCGTTCCATTATACC
ATCAGAACTGTCTGGTTGCTGTTAAAATCACCGAGCTTGTAATCCGTTATCGCCTGA
TTAAGTCTAGGTCGCTTAATATAAGGTGGTTCTCCCCTATACTACTCTCCATCTTATC
AGCCATTTAGTTTTGCTCTGGCTTCTTACTACCATCCAGTGTATAGTTACTTCTAATG
TCTCCACGTGAGCATTGCATCTAATTCCCCCAACGTTACCCCAACTGTA

>Hap_55 Panthera leo Mitogenomic Polymorphic Sites KEN_142
ACTTTCCGTCCCTCATCGGTAGTCATGCTTTACTCTAATGAGCGCGTTCCATTATACC
ATCAGAACTGTCTGGTTGCTGTTAAAATCACCGAGCTTGTAATCTGTTATCGCCTGAT
TAGGTCTAGGTCGCTTAATATAAGGTGGTTCTCCCCTGTACTACTCTCCATCTCATCA
GCCATTTAGTTTTGCTCTGGCTTCTTACTACCATCCAGCGTATAGTTACTTCTAATGT
CTCCACGTGAGCATTGCATCTAATTCCCCCAACGTTGCCCCAACTGTA

>Hap_56 Panthera leo Mitogenomic Polymorphic Sites TAN_081
ACTTTCCGTCCCTCATCGGTGGCCATGCTTTACTCTAATGAGCGCGTTCCATTATACC
ATCAGAACTGTCTGGTTGCTGTTAAAATCACCGAGCTTGTAATCTGTTATCGCCTGAT
TAAGTCTAGGTCGCTTAATATAAGGTGGTTCTCCCCTGTACTACTCTCCATCTTATCA
GCCATTTAGTTTTGCTCTGGCTTCTTACTACCATCCAGTGTATAGTCACTTCTAATGT
CTCCACGTGAGCATTGCATCTAATTCCCCCAACGTTACCCCAACTGTA

>Hap_57 Panthera leo Mitogenomic Polymorphic Sites TAN_153
ACTTTCCGTCCCTCATCGGTAGTCATGCTTTACTCTAATGAGCGCGTTCCATTATACC
ATCAGAACTGTCTGGTTGCTGTTAAAATCACCGAGCTTGTAATCTGTTATCGCCTGAT
TAAGTCTAGGTCGCTTAATATAAGGTGGTTCTCCCCTGTACTACTCTCCATCTTATCA
GCCATTTAGTTTTGCTCTGGCTTCTTACTACCATCCAGTGTATAGTTACTTCTAATGT
CTCCACGCGAGCATTGCATCTAATTCCCCCAACGTTACCCCAACTGTA

>Hap_58 Panthera leo Mitogenomic Polymorphic Sites TAN_107
ACTTTCCGTCCCTCATCGGTAGTCATGCTTTACTCTAATGAGCGCGTTCCATTATACC
ATCAGAACTGTCTGGTTGCTGTTAAAATCACCGAGCTTGTAATCTGTTATCGCCTGAT
TAAGTCTAGGTCGCTTAATATAAGGTGGTTCTCCCCTATACTACTCTCCATCTTATCA
GCCATTTAGTTTTGCTCTGGCTTCTTACTACCATCCAGTGTATAGTTACTTCTAATGT
CTCCACGTGAGCATTGCATCTAATTCCCCCAACGTTGCCCCAACTGTA

>Hap_59 Panthera leo Mitogenomic Polymorphic Sites TAN_087
ACTTTCCGTCCCTCATCGGTAGTCATGCTTTACTCTAATGAGCGCGTTCCATTATACC
ATCAGAACTGTCTGGTTGCTGTTAAAATCACCGAGCTTGTAATCTGTTATCGCCTGAT
TAAGTCTAGGTCGCTTAATATAAGGTGGTTCTCCCCTGTACTACTCTCCATCTTATCA
GCCATTTAGTTTTGCTCTGGCTTCTTACTACCATCCAGTGTATAGTCACTTCTAATGT
CTCCACGCGAGCATTGCATCTAATTCCCCCAACGTTGCCCCAACTGTA

>Hap_60 Panthera leo Mitogenomic Polymorphic Sites TAN_086
ACTTTCCGTCCCTCATCGGTAGTCATGCTTTACTCTAATGAGCGCGTTCCATTATACC
ATCAGAACTGTCTGGTTGCTGTTAAAATCACCGAGCTTGTAATCTGTTATCGCCTGAT
TAAGTCTAGGTCGCTTAATATAAGGTGGTTCTCCCCTGTACTACTCTCCATCTTATCA
GCCATTTAGTTTTGCTCTGGCTTCTTACTACCATCCAGTGTATAGTTACTTCTAATGT
CTCCACGTGAGCATTGCATCTAATTCCCCCAACGTTACCCCAACTGTA

>Hap_61 Panthera leo Mitogenomic Polymorphic Sites KEN_118
ACTTTCCGTCCCTCATCGGTGGTCATGCTTTACTCTAATGAGCGCGTTCCATTATACC
ATCAGAACTGTCTGGTTGCTGTTAAAATCACCGAGCTTGTAATCTGTTATCGCCTGAT
TAAGTCTAGGTCGCTTAATATAAGGTGGTTCTCCCCTGTACTACTCTCCATCTTATCA
GCCATTTAGTTTTGCTCTGGCTTCTTACTACCATCCAGTGTATAGTCACTTCTAATGT
CTCCACGTGAGCATTGCATCTAATTCCCCCAACGTTGCCCCAACTGTA

>Hap_62 Panthera leo Mitogenomic Polymorphic Sites KEN_116, TAN_111
ACTTTCCGTCCCTCATCGGTAGTCATGCTTTACTCTAATGAGCGCGTTCCATTATACC
ATCAGAACTGTCTGGTTGCTGTTAAAATCACCGAGCTTGTAATCTGTTATCGCCTGAT
TAAGTCTAGGTCGCTTAATATAAGGTGGTTCTCCCCTGTACTACTCTCCATCTTATCA
GCCATTTAGTTTTGCTCTGGCTTCTTACTACCATCCAGTGTATAGTCACTTTTAATGT
CTCCACGTGAGCATTGCATCTAATTCCCCCAACGTTACCCCAACTGTA

>Hap_63 Panthera leo Mitogenomic Polymorphic Sites KEN_115
ACTTTCCGTCCCTCATCGGTAGTCATGCTTTACTCTAATGAGCGCGTTCCATTATACC
ATCAGAACTGTCTGGTTGCTGTTAAAATCACCGAGCTTGTAATCCGTTATCGCCTGA
TTAAGTCTAGGTCGCTTAATATAAGGTGGTTCTCCCCTGTACTACTCTCCATCTTATC
AGCCATTTAGTTTTGCTCTGGCTTCTTACTACCATCCAGTGTATAGTCACTTCTAATG
TCTCCACGTGAGCATTGCATCTAATTCCCCCAACGTTGCCCCAACTGTA

>Hap_64 Panthera leo Mitogenomic Polymorphic Sites KEN_024, TAN_089
ACTTTCCGTCCCTCATCGGTAGTCATGCTTTACTCTAATGAGCGCGTTCCATTATACC
ATCAGAACTGTCTGGTTGCTGTTAAAATCACCGAGCTTGTAATCTGTTATCGCCTGAT
TAAGTCTAGGTCGCTTAATATAAGGTGGTTCTCCCCTGTACTACTCTCCATCTTATCA
GCCATTTAGTTTTGCTCTGGCTTCTTACTACCATCCAGTGTATAGTTACTTCTAATGT
CTCCACGTGAGCATTGCATCTAATTCCCCCAACGTTGCCCCAACTGTA

>Hap_65 Panthera leo Mitogenomic Polymorphic Sites KEN_019, KEN_020, TAN_079, TAN_083, TAN_106, TAN_141
ACTTTCCGTCCCTCATCGGTAGTCATGCTTTACTCTAATGAGCGCGTTCCATTATACC
ATCAGAACTGTCTGGTTGCTGTTAAAATCACCGAGCTTGTAATCTGTTATCGCCTGAT
TAAGTCTAGGTCGCTTAATATAAGGTGGTTCTCCCCTGTACTACTCTCCATCTTATCA
GCCATTTAGTTTTGCTCTGGCTTCTTACTACCATCCAGTGTATAGTCACTTCTAATGT
CTCCACGTGAGCATTGCATCTAATTCCCCCAACGTTACCCCAACTGTA

>Hap_66 Panthera leo Mitogenomic Polymorphic Sites KEN_021, KEN_022, KEN_023, KEN_025, KEN_117, KEN_154, KEN_KP498, TAN_080, TAN_084, TAN_088, TAN_123, TAN_124, TAN_125, TAN_126
ACTTTCCGTCCCTCATCGGTAGTCATGCTTTACTCTAATGAGCGCGTTCCATTATACC
ATCAGAACTGTCTGGTTGCTGTTAAAATCACCGAGCTTGTAATCTGTTATCGCCTGAT
TAAGTCTAGGTCGCTTAATATAAGGTGGTTCTCCCCTGTACTACTCTCCATCTTATCA
GCCATTTAGTTTTGCTCTGGCTTCTTACTACCATCCAGTGTATAGTCACTTCTAATGT
CTCCACGTGAGCATTGCATCTAATTCCCCCAACGTTGCCCCAACTGTA

>Hap_67 Panthera leo Mitogenomic Polymorphic Sites RSA_061
ATTTCCCGCCCTTCATTGGCAACCACACTTCACTTTAATGAGCGCATCCCACTGTGTC
ATCAAAACTGTTTGGCTATTGTTAAGACCACCGAGGTTATAATCCGTTACCGCTTGA
TTAGGTCCGGGCTGCTTAGCGTAGGACGATTCTTCCTCGTACTACCCCCTATCTCGCC
AGCCGTTCAACTTTGCCCTAGCTTCTTACCACCACCTAGCGTGTAACCACTTCCAGTG
CCTTCACGTGAGCACCGTATCCAACCTCCTCAACGTTACCCCGGTTATA

>Hap_68 Panthera leo Mitogenomic Polymorphic Sites RSA_060, RSA_062
ACTTCCCGCCCTTCATTGGCAACCACACTTCACTTTAACAGGCGCATCCCACTGTGTC
ATCAAAACTGTTTGGCTATTGTTAAGACCACCGAGGTTATAATCCGTTACCGCTTGA
TTAGGTTCGGGCTGCTTAGCGTAGGACGATTCTTCCTCGTACTACCCCCTATCTCGCC
AGCCGTTCAACTTTGCCCTAGCTTCTTACCACCACCTAGCGTGTAACCACTTCCAGTG
CCTTCACGTGAGCACCGTATCCAACCTCCTCAACGTTACCCCGGTTATA

>Hap_69 Panthera leo Mitogenomic Polymorphic Sites RSA_059
ACTTCCCGCCCTTCATTGGCAACCACACTTCACTTTAACAGGCGCATCCCACTGTGTC
ATCAAAACTGTTTGGCTATTGTTAAGACCACCGAGGTTATAATCCGTTACCGCTTGA
TTAGGTTCGGGCTGCTTAGCGTAGGACGATTCTTCCTCGTACTACCCCCTATCTCGCC
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CCTTCACGTGAGCACCGTATCCAACCTCCTCAGCACTATCCCGGTTATA

>Hap_70 Panthera leo Mitogenomic Polymorphic Sites RSA_066
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ATCAAAACTGTTTGGCTATTGTTAAGACCACCGAGGTTATAATCCGTTACCGCTTGA
TTAGGTTCGGGCTGCTTAGCGTAGGACGATTCTTCCTCGTACTACCCCCTATCTCGCC
AGCCGTTCAACTTTGCCCTAGCTTCTTACCACCACCTAGCGTGTAACCACTTCCAGTG
CCTTCACGTGAGCACCGTATCCAACCTCCTCAGCACTATCCCGGTTATA

>Hap_71 Panthera leo Mitogenomic Polymorphic Sites RSA_KP500
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ATCAAAACTGTTTGGCTATTGTTAAGACCACCGAGGTTATAATCCGTTACCGCTTGA
TTAGGTTCGGGCTGCTTAGCGTAGGACGATTCTTCCTCGTACTACCCCCTATCTCGCC
AGCCGTTCAACTTTGCCCTAGCTTCTTACCACCACCTAGCGTGTAACCACTTCCAGTG
CCTTCACGTGAGCACCGTATCCAACCTCCTCAGCACTATCCCGGTTATA

>Hap_72 Panthera leo Mitogenomic Polymorphic Sites BOT_001
ACTTTCCGCTCTTCGTCGGTAACCATGCTTTACTTTAATGAGCGCGTCCCACTATACC
ATCAAAACTGTTTGGTTATTGTTAAGATCACCGAGGTCATAATCCGTTACCGCCTGA
TTAGGTTCGAGCTGCTTAACATAAGACGATTCTTCCTTGTACTACCCTCTATCTCGTC
AGCCATTTAGCTTTGCTCTAGCTTCTTACTACCATCCAGCGTGTAGTCACTTCCGATA
TCTCCACGTAAGCATTGTATCCAACCTCCTCAATACTATCCCAACTGTA

>Hap_73 Panthera leo Mitogenomic Polymorphic Sites BOT_069
ACTTTCCGCTCTTCATTGGTAACCATGCCTCACTTTAATGAGCGCGTCCCACTATGTC
ATCAGAACCGTTTGGTTATTGTTAAGACCACCGAGGCCATAATCCGTTATCGCCTGA
TTAGGTTCGAGCCGCTTAGCATAGGACAATTCTTCCTTGCACTACCCCCTACCTCGTT
AGCCATTCAATTTTGCTCTGGCTTCCTACTACCACCTAGCGTGTAGTCACTTCCGATA
TCTTCACGTAAGCATTGCATCCAACCTCCCCAATACTATCCCAACTGTA

>Hap_74 Panthera leo Mitogenomic Polymorphic Sites NAM_KP496
ACTTTCCGCCCTTCATTGGTAGCCATACTTCACTTTAACAAGCGCGTCCCACTGTGTC
ATCAAAACTGTCTGGCTATTGTTAAGACCACCGAGGTTATAATCCGTTACCGCCTGA
TTAGGTTCGGGCTGCTTAGCATAGGACGATTCTTCCTCGTACTATCCCCTATCTCGCC
AGCCATTCAACTTTGCCCTAACTTCTTACTACCACCTAGCGTGTAGTCACTTCTAATG
TTTTCACGTGAGTATTGTATCCAACCTCCTCAATACTATCCCGGCCGTA

>Hap_75 Panthera leo Mitogenomic Polymorphic Sites RSA_132
ATTTTCCGCTCTTCGTTGGTAACCATACCTCACTTTAACAAGCGCGTCCCACTGTGTC
ATCAAAACTGTTTGGCTATTGTTAAAACCACCGAGGCCATAATCCGTTACCGCCTGA
TTAGGTCTGAGCTGCTTAGCATAGGGCGATTCTTCCTCGTACTACCCCCTACCTCGCC
AGCCATTCAACTTTGTCCTAGCTTCCTACTACCACCTAGCGTGTAGTCACTTCCGATA
TCTTCACGTAAGCATTGTATCCAACCTCCTCAATACTATCCCGGCTGTA

>Hap_76 Panthera leo Mitogenomic Polymorphic Sites ZIM_010
ACTTTCCGCTCTTCGTTAGTAACCATACCTCACTTTAACAAGCGCGTCCCACTGTGTC
ATCAAAACTGTTTGGCTATTGTTAAGACCACCGAGGCCATAATCCGTTACCGCCTGA
TTAGGTTCGAGCTGCTTAGCATAGGACGATTCTTCCTCGTACTACCCCCTACCTCGCC
AGCCATTCAACTTTGTCCTAGCTTCCTACTACCACCTAGCGTGTAGTCACTTCCGATA
TCTTCACGTAAGCATTGTATCCAACCTCCTCAACGTTACCCCGGCTGTA

>Hap_77 Panthera leo Mitogenomic Polymorphic Sites COG_003, GAB_004
ACTTTCCGCTCTTCGTTGGTAACCATACCTCACTTTAACAAGCGCGTCCCACTGTGTC
ATCAAAACCGTTTGGCTATTGTTAAGACCACCGAGGCCATAATCCGTTACCGCCTGA
TTAGGTTCGAGCTGCTTAGCATAGGACGATTCTTCCTCGCACTACCCCCTACCTCGCC
AGCCATTCAACTTTGTCCTAGCTTCCTACTACCACCTAGCGTGTAGTCACTTCCGATA
TCTTCACGTAAGTATTGTATCCAACCTCCTCAATACTATCCCGGCTGTA

>Hap_78 Panthera leo Mitogenomic Polymorphic Sites BOT_071
ACTTTCCGCTCTTCGTTGGTAACCATACCTCACTTTAACAAGCGCGTCCCACTGTGTC
ATCAAAACTGTTTGGCTATTGTTAAGACCACCGAGGCCATAATCCGTTACCGCCTGA
TTAGGTTCGAGCTGCTTAGCATAGGACGATTCTTCCTCGCACTACCCCCTACCTCGCC
AGCCATTCAACTTTGTCCTAGCTTCCTACTACCACCTAGCGTGTAGTCACTTCCGATA
TCTTCACGTAAGCATTGTATCCAACCTCCTCAATACTATCCCGGCTGTA

>Hap_79 Panthera leo Mitogenomic Polymorphic Sites BOT_129, BOT_131
ACTTTCCGCTCTTCGTTGGTAACCATACCTCACTTTAACAAGCGCGTCCCACTGTGTC
ATCAAAACTGTTTGGCTATTGTTAAGACCACCGAGGCCATAATCCGTTACCGCCTGA
TTAGGTTCGAGCTGCTTAGCATAGGACGATTCTTCCTCGTACTACCCCCTACCTCGCC
AGCCATTCAACTTTGTCCTAGCTTCCTACTACCACCTAGCGTGTAGTCACTTCCGATA
TCTTCACGTAAGCATTGTATCCAACCTCCTCAATACTATCCCGGCTGTA

>Hap_80 Panthera leo Mitogenomic Polymorphic Sites BOT_073
ATTTTCCGCTCTCCGTTGGTAACCATGCCTCACTTTAATGAGCGCGTCCCACTGTGTC
ATCAAAACTGTTTGGTTATTGTTAAGACCACCGAGGTCATAATCCGTTACCGCCTGA
TTAGGTTCGAACTGCTTAGCATAGGACGATTCTTCCTCGCACTACCCCCTACCTCGTC
AGCCATTCAACTTTGTCCTAGCTTCCTACTACCACCTAGCGTGTAGTCATTTCCGATA
TCTTCACGTGAGCATTGTATCCAACCTCCTCAACGTTACCCCGGCTGTA

>Hap_81 Panthera leo Mitogenomic Polymorphic Sites BOT_074
ACTTTCCGCTCTCCGTTGGTAACCATGCCTCACTTTAATGAGCGCGTCCCACTGTGTC
ATCAAAACTGTTTGGCTATTGTTAAGACCACCGAGGTCATAATCCGTTACCGCCTGA
TTAGGTCCGAACTGCTTAGCATAGGACGATTCTTCCTCGTACTACCCCCTACCTCGTC
AGCCATTCAACTTTGTCCTAGCTTCCTACTACCACCTAGCGTGTAGTCATTTCCGATA
TCTTCACGTAAGCATTGTATCCAACCTCCTCAATACTATCCCGGCTGTA

>Hap_82 Panthera leo Mitogenomic Polymorphic Sites BOT_133
ACTTTCCGCTCTCCGTTGGTAACCATACCTCACTTTAACAAGTGCGTCCCACTGTGTC
ATCAAAACTGTTTGGCTATTGTTAAGACCACCGAGGTCATAATCCGTTACCGCCTGA
TTAGGTCTGAACTGCTTAGCATAGGACGATTCTTCCTCGTACTACCCCCTACCTCGCC
AGCCATTCAACTTTGTCCTAGCTTCCTACTACCACCTAGCGTGTAGTCATTTCCGATA
TCTTCACGTAAGCATTGTATCCAACCTCCTCAATACTATCCCGGCTGTA

>Hap_83 Panthera leo Mitogenomic Polymorphic Sites BOT_002
ACTTTCCGCTCTCCGTTGGTAACCATACCTCACTTTAACAAGCGCGTCCCACTGTGTC
ATCAAAACTGTTTGGCTATTGTTAAGACCACCGAGGTCATAATCCGTTACCGCCTGA
TTAGGTCCGAACTGCTTAGCATAGGACGATTCTTCCTCGTACTACCCCCTACCTCGCC
AGCCATTCAACTTTGTCCTAGCTTCCTACTACCACCTAGCGTGTAGTCATTTCCGATA
TCTTCACGTAAGCATTGTATCCAACCTCCTCAATACTATCCCGGCTGTA

>Hap_84 Panthera leo Mitogenomic Polymorphic Sites BOT_137
ACTTTCCGCTCTCCGTTGGTAACCATACCTCACTTTAACAAGCGCGTCCCACTGTGTC
ATCAAAACTGTTTGGCTATTGTTAAGACCACCGAGGTCATAATCCGTTACCGCCTGA
TTAGGTTCGAACTGCTTAGCATAGGACGATTCTTCCTCGTACTACCCCCTACCTCGCC
AGCCATTCAACTTTGTCCTAGCTTCCTACTACCACCTAGCGTGTAGTCATTTCCGATA
TCTTCACGTAAGCATTGTATCCAACCTCCTCAATACTATCCCGGCTGTA

>Hap_85 Panthera leo Mitogenomic Polymorphic Sites BOT_076
ATTTTCCGCTCTCCGTCGGTAACCATGCCTCACTTTAATGAGCGCGTCCCACTGTGTC
ATCAAAACTGTTTGGCTATTGTTAAGACCACCGAGGTCATAATCCGTTACCGCCTGA
TTAGGTTCGAACTGCTTAGCATAGGACGATTCTTCCTCACACTACCCCCTACCTCGCC
AGCCATTCAACTTTGTCCTAGCTTCCTACTACCACCTAGCGTGTAGTCATTTCCGATA
TCTTCACGTAAGCATTGTATCCAACCTCCTCAATACTATCCCGGCTGTA

>Hap_86 Panthera leo Mitogenomic Polymorphic Sites NAM_KP504
ATTTTCCGCTCTTCGTTGGTAACCATACCTCACTTTAACAAGTGCGTCCCACTGTGTC
ATCAAAACTGTTTGGCTATTGTTAAGACCACCGAGGTCATAATCCGTTACCGCCTGA
TTAGGTTCGAACTGCTTAGCATAGGACGATTCTTCCTCGCACTACCCCCTACCTCGCC
AGCCATTCAACTTTGTCCTAGCTTCCTACTACCACCTAGCGTGTAGTCACTTCCGATA
TCTTCACGTAAGCATTGTATCCAACCTCCTCAATACTATCCCGGCTGTA

>Hap_87 Panthera leo Mitogenomic Polymorphic Sites BOT_075
ATTTTCCGCTCTCCGTTGGTAACCATACCTCACTTTAACAAGCGCGTCCCACTGTATC
ATCAAAACTGTTTGGCTATTGTTAAGACCACCGAGGTCATAATCCGTTACCGCCTGA
TTAGGTCCGAACTGCTTAGCATAGGACGATTCTTCCTCGTACTACCCCCTACCTCGCC
AGCCATTCAACTTTGTCCTAGCTTCCTACTACCACCTAGCGTGTAGTCATTTCCGATA
TCTTCACGTAAGCATTGTATCCAACCTCCTCAATACTATCCCGGCTGTA

>Hap_88 Panthera leo Mitogenomic Polymorphic Sites BOT_070
ATTTTCCGCTCTCCGTTGGTAACCATACCTCACTTTAACAAGCGCGTCCCACTGTGTC
ATCAAAACTGTTTGGCTATTGTTAAGACCACCGAGGTCATAATCCGTTACCGCCTGA
TTAGGTCCGAACTGCTTAGCATAGGACGATTCTTCCTCGCACTACCCCCTACCTCGC
CAGCCATTCAACTTTGTCCTAGCTTCCTACTACCACCTAGCGTGTAGTCATTTCCGAT
ATCTTCACGTAAGCATTGTATCCAACCTCCTCAATACTATCCCGGCTGTA

>Hap_89 Panthera leo Mitogenomic Polymorphic Sites BOT_077
ATTTTCCGCTCTCCGTTGGTAACCATACCTCACTTTAACAAGCGCGTCCCACTGTGTC
ATCAAAACTGTTTGGCTATTGTTAAGACCACCGAGGTCATAATCCGTTACCGCCTGA
TTAGGTTCGAACTGCTTAGCATAGGACGATTCTTCCTCGCACTACCCCCTACCTCGCC
AGCCATTCAACTTTGTCCTAGCTTCCTACTACCACCTAGCGTGTAGTCATTTCCGATA
TCTTCACGTAAGCATTGTATCCAACCTCCTCAATACTATCCCGGCTGTA

APPENDIX C

SUPPLEMENTAL INFORMATION

C1: DOMESTIC CAT MICROSATELLITES USED IN LION RESEARCH

Loci	1	2	3	4	5	6	7	8	9	10	11	12	13	Total
F 115						x		x	x					3
F 37						x								1
F 41						x								1
F 42						x	x							2
FCA 001			x			x	x	x	x	x				6
FCA 005			x											1
FCA 006	x		x					x					x	4
FCA 007			x											1
FCA 008	x		x			x	x	x	x	x			x	8
FCA 014	x		x	x	x			x						5
FCA 023			x		x	x								3
FCA 026		x	x	x	x	x	x		x	x			x	9
FCA 030			x	x	x									3
FCA 031						x	x	x	x	x	x		x	7
FCA 032		x	x									x		3
FCA 036			x											1
FCA 039			x											1
FCA 043			x		x									2
FCA 044			x											1
FCA 045			x	x	x			x	x	x			x	7
FCA 048			x											1
FCA 051			x											1
FCA 052			x											1
FCA 057		x	x			x	x							4
FCA 058			x											1
FCA 059			x											1
FCA 066			x											1
FCA 069	x		x			x		x			x			5
FCA 075		x	x			x	x				x	x		6
FCA 077			x	x	x			x	x	x		x	x	8
FCA 078			x											1
FCA 080			x											1
FCA 081			x											1
FCA 082			x											1
FCA 085	x	x	x			x	x	x			x		x	8
FCA 088a			x											1
FCA 090			x											1
FCA 091	x	x	x					x					x	5
FCA 094			x	x	x							x		4
FCA 096			x	x	x	x	x				x		x	7
FCA 097		x	x			x	x					x		5
FCA 098	x		x										x	3
FCA 100			x											1
FCA 105	x		x			x		x						4
FCA 107			x											1
FCA 113						x	x				x			3
FCA 126	x	x	x	x	x	x	x	x	x	x	x	x	x	13
FCA 129	x		x					x						3
FCA 132			x	x	x									3
FCA 133			x											1
FCA 136		x	x											2
FCA 139	x		x					x						3
FCA 140			x											1
FCA 144		x	x											2

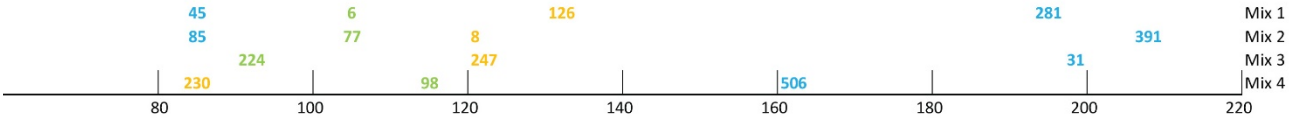
Loci	1	2	3	4	5	6	7	8	9	10	11	12	13	Total
FCA 149			x											1
FCA 153			x											1
FCA 159			x											1
FCA 161		x	x											2
FCA 166			x											1
FCA 171			x											1
FCA 176			x											1
FCA 178		x	x											2
FCA 187			x	x	x									3
FCA 191		x	x	x	x									4
FCA 192			x											1
FCA 193			x			x	x							3
FCA 200			x											1
FCA 201			x											1
FCA 205	x	x	x		x			x				x		6
FCA 208	x	x	x					x				x		5
FCA 210			x											1
FCA 211	x	x	x											3
FCA 212			x											1
FCA 214			x											1
FCA 221			x					x						2
FCA 224	x	x	x			x	x	x				x	x	8
FCA 229	x		x					x						3
FCA 230	x		x			x	x	x					x	6
FCA 231			x											1
FCA 240			x			x	x							3
FCA 247	x	x	x					x				x	x	6
FCA 249			x											1
FCA 254			x											1
FCA 261			x											1
FCA 262			x											1
FCA 272						x	x	x	x					4
FCA 275		x	x			x	x	x	x		x	x		8
FCA 280			x											1
FCA 281	x		x										x	3
FCA 290			x											1
FCA 293			x											1
FCA 298			x											1
FCA 304			x											1
FCA 310			x			x					x			3
FCA 311			x											1
FCA 322			x											1
FCA 327			x											1
FCA 339			x											1
FCA 343			x											1
FCA 344														0
FCA 391	x					x	x	x	x				x	6
FCA 441	x					x		x						3
FCA 453						x	x				x			3
FCA 506						x	x	x	x	x	x		x	7
FCA 567								x	x	x				3
FCA 628						x	x	x	x					4
FCA 651						x								1
# Loci Used	21	19	92	11	14	31	22	29	14	9	11	11	17	107

1: Antunes et al 2008 (N=357), **2**: Bertola et al 2015 (N=48), **3**: Driscoll et al 2002 (N=60), **4**: Dubach et al 2013 (N=480), **5**: Lyke et al 2013 (N=90), **6**: Miller et al 2014 (N=361), ***7****: Miller et al 2015 (N=351),* **8:** Morandin et al 2014 (N=157), **9**: Spong et al 2002 (N=70), **10**: Tende et al 2014 (N=18), ***11****: Tensen et al 2018 (N=42),* ***12****: van Hooft et al 2018 (N=97)* and **13**: this study (N=30).
Studies in *italics* were not used for primer selection because they were not yet available or duplicate data.

C2: Multiplex Design – Final Primer Combinations

Desired Primer Combinations

Primer	Chrom.	Length	Dye	Primer	Chrom.	Length	Dye	Primer	Chrom.	Length	Dye	Primer	Chrom.	Length	Dye	
045	D4	86	6-FAM	**006**	D3	108	VIC	**126**	B1	132	NED	**281**	E1	199	6-FAM	Mix 1
085	A2	85	6-FAM	**077**	C2	107	VIC	**008**	A1	**136**	NED	**391**	B3	195	6-FAM	Mix 2
				224	A3	93	VIC	**247**	C1	**126**	NED	**031**	E3	201	6-FAM	Mix 3
230	B3	**87**	6-FAM	**098**	A2	115	VIC					**506**	F2	**162**	6-FAM	Mix 4



C3: STR Calibration Results

		006		008		031		045		077		085		098		126		224		230		247		281		391		506	
BEN	Leo	122	136	113	131	198	198	80	96	106	106	86	92	102	102	125	127	92	94	86	86	114	126	223	223	178	178	171	191
	Predicted	204	218	126	144			132	148	148	148	129	135	104	104	137	139	166	168	92	92	135	147	242	242				
	FCA			126	144			132	148	148	148	129	135	104	104	137	139	168	170			135	147	243	243				
	FCA			126	144			132	148	148	148	129	135	104	104	137	139	168	170			135	147	243	243				
	Study											145	151			155	157	184	186			160	172						
	Leo Diff											59	59			30	30	92	92			46	46						
	FCA Diff											16	16			18	18	16	16			25	25						

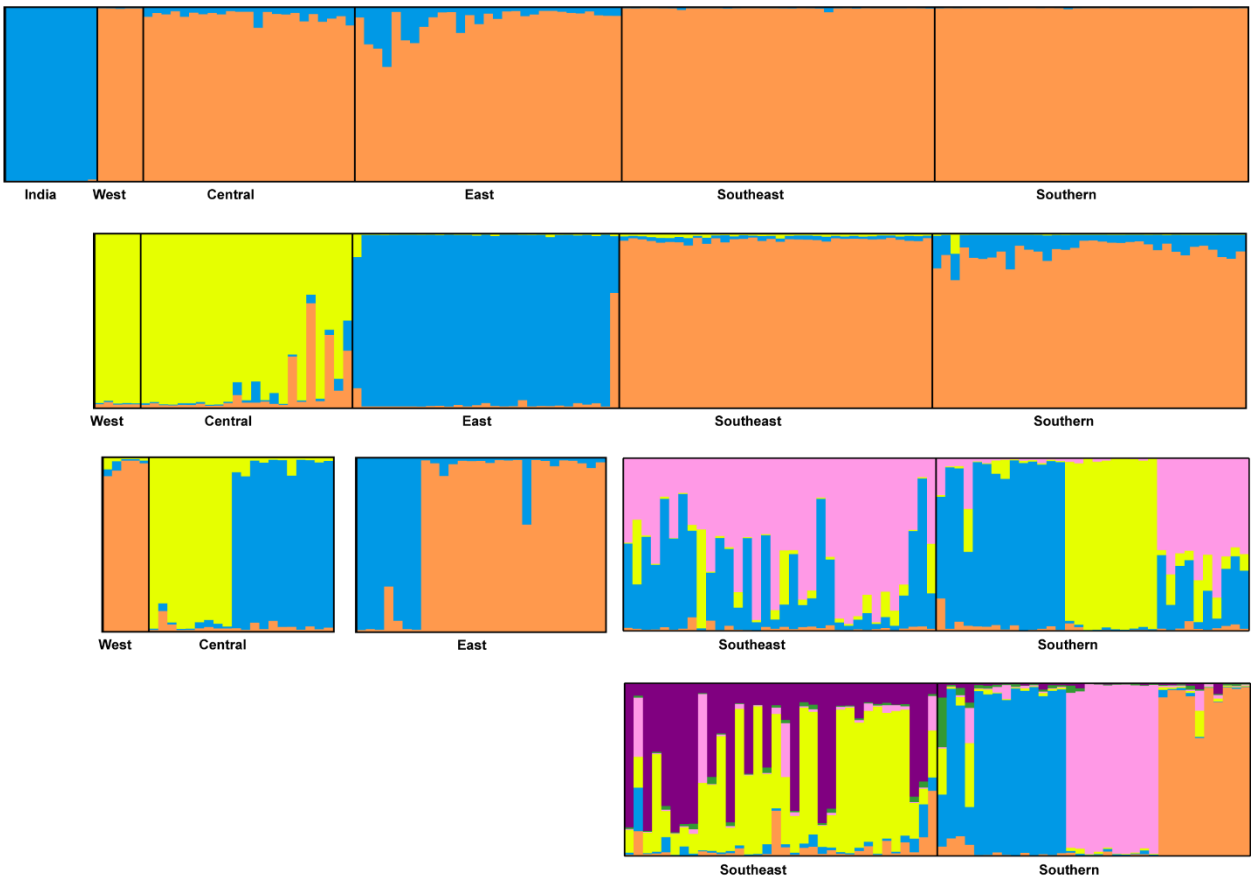
CAM	Leo	110	136	111	111	194	198	80	100	104	112	80	80	92	102	137	139	78	78	86	86	126	128	225	225	182	182	187	187
	Predicted	192	218	124	124			132	152	146	154	123	123	94	104	149	151	152	152	92	92	147	149	244	244				
	FCA			122	122			132	154	146	154	123	123	96	106	148	150	150	150	92	92	147	149	145	145				
	FCA			122	122			132	154	146	154	123	123	96	104	148	150	152	152	92	92	147	149	145	145				
	Study											141	141			167	169	170	170			172	174						
	Leo Diff											61	61			30	30	92	92			46	46						
	FCA Diff											18	18			19	19	20	20			25	25						

254	Leo	94	104	113	131	192	196	80	80	98	102	80	94	102	102	125	129	86	86	80	88	122	122	211	229	194	198	193	197
	FCA	176	186	126	144			132	132	140	144	123	137	104	104	137	141	160	160	86	94	143	143	230	248				
	Diff	82	82	13	13			52	52	42	42	43	43	2	2	12	12	74	74	6	6	21	21	19	19				
387	Leo	104	104	127	127	196	196	80	80	102	104	80	80	104	108	131	143	78	86	78	82	114	116	213	217	174	190	191	193
	FCA	186	186	140	140			132	132	144	146	123	123	106	110	143	155	152	160	84	88	135	137	232	236				
	Diff	82	82	13	13			52	52	42	42	43	43	2	2	12	12	74	74	6	6	21	21	19	19				
	Diff	82	82	13	13			52	52	42	42	43	43	2	2	12	12	74	74	6	6	21	21	19	19				

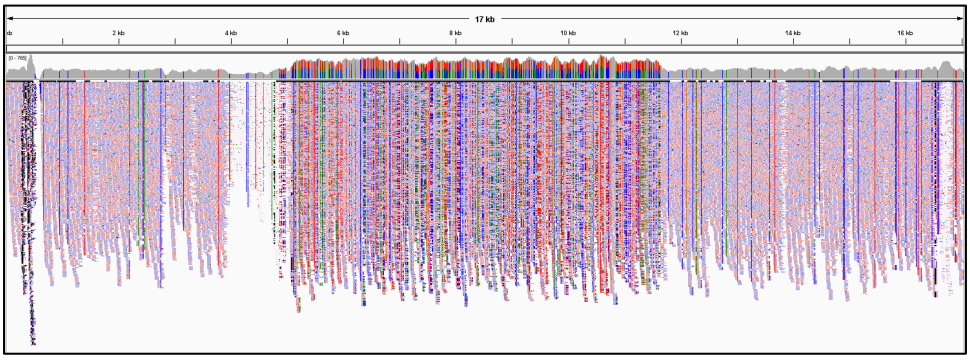
Driscol	MCA	192	192	126	126			133	133	150	150	124	124	111	111	140	140	173	173	101	101	140	140	123	123				
	Leo MCA	124	124	111	111	196	196	80	80	104	104	80	80	102	102	125	125	86	86	88	88	128	128	213	213	174	174	193	193
	Diff	68	68	15	15			53	53	46	46	44	44	9	9	15	15	87	87	13	13	12	12	-90	-90				
	Cal Used	84		13				53		44		44		7		13		77		13		26		-90					

Allele call results from amplification with FCA and Leo primers compared to published allele calls to determine a correction to calibrate allele calls across studies.

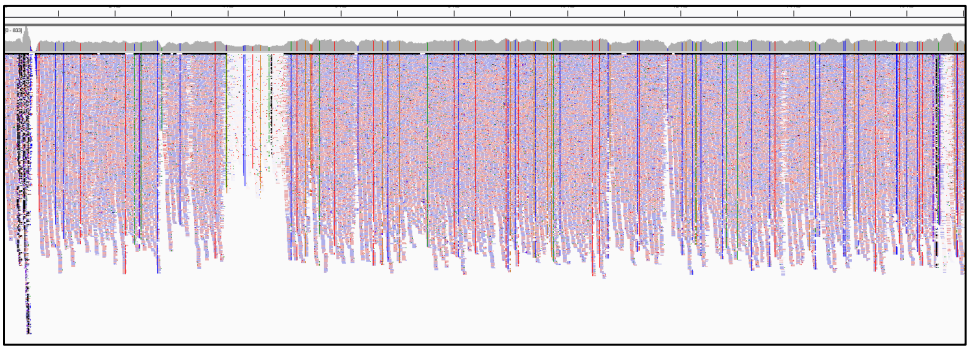
C4: STEP-BY-STEP HIERARCHICAL STRUCTURE RESULTS



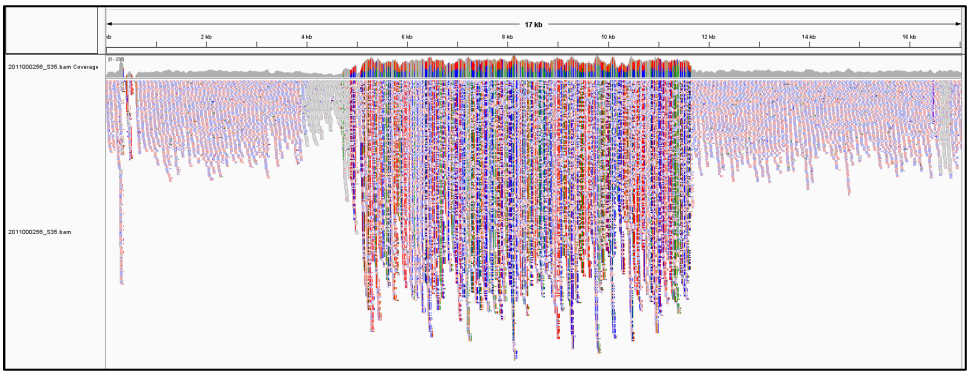
C5: MITOGENOME ALIGNMENTS WITH AND WITHOUT NUMT CORRECTION



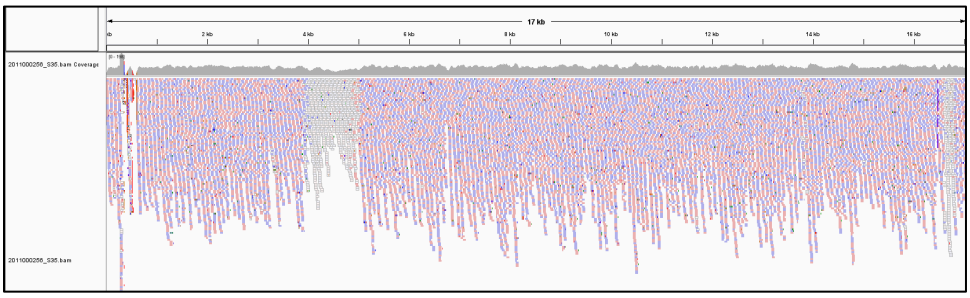
Historical Lion AMNH_81840 without numt correction



Historical Lion AMNH_81840 with numt correction

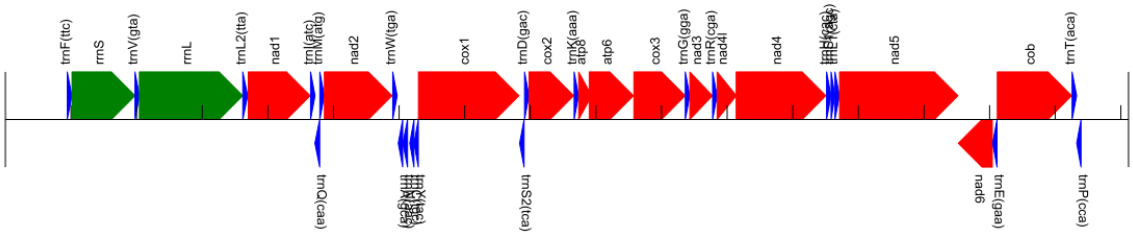


Modern Lion 2011000256 without numt correction



Modern Lion 2011000256 with numt correction

C6: ANNOTATION OF LION MITOGENOME



Abbreviation	Name	Seq	Direction	Start	End
tRNA-Phe	Phenylalanine	trnF(ttc)	+	943	1015
12S	ribosomal RNA	rrnS	+	1015	1977
tRNA-Val	Valine	trnV(gta)	+	1975	2044
16S	ribosomal RNA	rrnL	+	2042	3618
tRNA-Leu2	Leucine 2	trnL2(tta)	+	3618	3693
ND1	NADH dehydrogenase subunit 1	nad1	+	3701	4646
tRNA-Ile	Isoleucine	trnI(atc)	+	4651	4720
tRNA-Gln	Glutamine	trnQ(caa)	-	4717	4791
tRNA-Met	Methionine	trnM(atg)	+	4792	4861
ND2	NADH dehydrogenase subunit 2	nad2	+	4861	5893
tRNA-Trp	Tryptophan	trnW(tga)	+	5903	5972
tRNA-Ala	Alanine	trnA(gca)	-	5987	6056
tRNA-Asn	Asparagine	trnN(aac)	-	6057	6130
tRNA-Cys	Cysteine	trnC(tgc)	-	6163	6228
tRNA-Tyr	Tyrosine	trnY(tac)	-	6228	6294
COI	cytochrome c oxidase subunit I	cox1	+	6295	7828
tRNA-Ser2	Serine 2	trnS2(tca)	-	7837	7906
tRNA-Asp	Aspartic acid	trnD(gac)	+	7912	7981
COX2	cytochrome c oxidase subunit II	cox2	+	7981	8662
tRNA-Lys	Lysine	trnK(aaa)	+	8668	8736
ATP8	ATP synthase F0 subunit 8	atp8	+	8737	8935
ATP6	ATP synthase F0 subunit 6	atp6	+	8898	9573
COX3	cytochrome c oxidase subunit III	cox3	+	9578	10361
tRNA-Gly	Glycine	trnG(gga)	+	10362	10431
ND3	NADH dehydrogenase subunit 3	nad3	+	10431	10776
tRNA-Arg	Arginine	trnR(cga)	+	10778	10847
ND4l	NADH dehydrogenase subunit 4L	nad4l	+	10847	11141
ND4	NADH dehydrogenase subunit 4	nad4	+	11137	12505
tRNA-His	Histidine	trnH(cac)	+	12515	12584
tRNA-Ser1	Serine 1	trnS1(agc)	+	12584	12643
tRNA-Leu1	Leucine 1	trnL1(cta)	+	12643	12713
ND5	NADH dehydrogenase subunit 5	nad5	+	12713	14519
ND6	NADH dehydrogenase subunit 6	nad6	-	14523	15042
tRNA-Glu	Glutamic acid	trnE(gaa)	-	15045	15114
cytb	cytochrome b	cob	+	15117	16251
tRNA-Thr	Threonine	trnT(aca)	+	16257	16327
tRNA-Pro	Proline	trnP(cca)	-	16327	16394
D-loops	control region				

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CT

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C7: Select Photos of Historical Lion Collection at Museums

From top left: Lion label from Carnegie Museum; Collecting samples at the American Museum; Differences in lion dentition; Tooth fragments from a cub; A lion paw; Various lion bones at Yale Peabody Museum; Cross section of a lion toe; Complete lion skeleton at the Carnegie Museum Holdings; Stack of lion bones at the American Museum.