Genetic diversity in fragmented southern African giraffe populations.

by

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This study is dedicated to my loving parents Heinrich and Therésa van Niekerk
for their unconditional love, support, and understanding,
as well as for encouraging me to follow my dreams.

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To whom it may concern

I, Marika Edna van Niekerk, declare that the Master's Degree research dissertation that I herewith submit for the Master's Degree qualification Genetics at the University of the Free State is my own independent work, and that I have not previously submitted it for a qualification at another institution of higher education. I further cede copyright of the dissertation in favour of the University of the Free State.

Marika Edna van Niekerk

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LIST OF ABBREVIATIONS AND SYMBOLS

Symbols used:

°C	Degrees Celsius
x g	G-force acceleration
µ	Micro: 10^{-6}
%	Percentage
®	Registered trademark
™	Trademark

Abbreviations used:

AFSp	All Free State populations
AMOVA	Analysis of Molecular Variance
B.C.	Before Christ
BLAST	Basic Local Alignment Search Tool
bp	Base pair
COI	Cytochrome oxidase I
CR	Control region
Cyt *b*	Cytochrome *b*
dH_2O	Distilled water
ddH_2O	Double distilled water
D_a	Average number of net nucleotide substitutions per site between Populations
D_{xy}	Average number of nucleotide substitutions per site between Populations
DNA	Deoxyribonucleic acid
dNTP	Deoxyribonucleotide triphosphate
et al.	*et alli*: and others
ETOH	ethanol
FS	Free State
FS DESTEA	Free State Department of Economic, Small Business

	Development, Tourism and Environmental Affairs
F_{ST}	Fixation Index
G. c.	*Giraffa camelopardalis*
h	Number of haplotypes
ha	Hectares
H_2O	Water
H_d	Haplotype diversity
H_o	Observed heterozygosity
HCl	Hydrochloric acid
HiDi	Highly deionized formamide
i.e.	*id est*: in other words, that is
IUCN	International Union for the Conservation of Nature
kg	Kilogram
km	Kilometre
km^2	Kilometre squared
m	Metres
mg	Milligram
ml	Millilitre
ML	Maximum Likelihood
mM	millimolar
mtDNA	Mitochondrial Deoxyribonucleic Acid
MNR	Municipal Nature Reserve
NADH	Nicotinamide adenine dinucleotide
NC	Northern Cape
NCBI	National Centre for Biotechnology Information
ng	Nanograms
ng/µl	Nanograms per microlitre
PCR	Polymerase chain reaction
PF	Private Farm
P_i	Nucleotide Diversity
pmol	Picomol

PNR	Provincial Nature Reserve
PWR	Private Wildlife Reserve
RFLP	restriction fragment length polymorphism
rpm	Revolutions per minute
SPFSp	Small Private Free State populations
STR	Short Tandem Repeat
TAE	Tris-acetate-EDTA buffer
µl	Microlitres
UV	Ultraviolet
v	Version
V	Volts

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ABSTRACT

The giraffe (*Giraffa camelopardalis*) is distributed throughout sub-Sahara in savannah habitat. It is currently listed as *Vulnerable* on the IUCN Red Data List, as their numbers are declining. Little is known about the genetic characteristics of giraffe in South Africa. This molecular analysis of the introduced giraffe populations in the Free State Province thus provides new insights into the species' population genetics across the Province. The specific aims of this study were to quantify the levels of genetic diversity within individual giraffe populations; and to determine the genetic structure of *Giraffa camelopardalis* in the Free State Province. For this purpose, a total of 129 faecal samples were taken from 20 populations within the Free State Province, and one population from the Northern Cape Province; and with reference sequences from all currently recognized sub-species taken from GenBank. Genetic diversity and genetic differentiation was quantified using sequence data from the Cyt *b* and D-loop mtDNA regions. Two haplotypes were identified for the Cyt *b* gene region, with 10 haplotypes identified for the D-loop region. Nucleotide diversity ranged from 0 to 0.132%. The results obtained indicated low levels of genetic diversity within isolated populations; however, there was more diversity present in the larger populations in comparison to the smaller populations, and even higher levels within pooled populations that can potentially be managed as a metapopulation. Various approaches to reconstruct relationships among populations, including Maximum Likelihood, a Bayesian approach and haplotype networks, showed very similar results. The results portrayed northern and southern groups when all samples and reference material were included, with individuals from the current study clustering with the southern clade. Population pairwise F_{ST} values and other measures of differentiation confirmed the strength. The extralimital giraffe population in Central South Arica was thus found to consist of more than one subspecies, with *G. c. angolensis* (or possibly *G. c. giraffa* x *G. c. angolensis* hybrids) surprisingly detected in a number of populations. Several recommendations were formulated in terms of the future management and conservation of giraffe in Nature Reserves and private game farms in the Free State Province. The most practical approach for dealing with inbreeding would evidently be to exchange

individuals between populations, but this should be supplemented by measures such as the implementation of a database for the Province and monitoring. A metapopulation approach to conserving genetic diversity is strongly recommended, since giraffe frequently occur in low numbers and this situation is unlikely to change. To enhance future studies, sequences of nuclear genes, as well as microsatellite markers should be added to supplement the current mtDNA-based data. Improved geographic coverage within South Africa, and specifically including naturally-occurring populations, would also be beneficial.

Key words: extralimital, fragmentation, *Giraffa camelopardalis*, genetic differentiation, genetic diversity, giraffe, metapopulation, mtDNA.

Chapter 1.

Introduction to Giraffe and Conservation Genetics

There is no available genetic information pertaining to the introduced giraffe population in the Free State Province, South Africa. This is the first study being done within Central South Africa to determine the levels of genetic diversity within individual giraffe populations in this area. The genetic structure of *Giraffa camelopardalis* (G. c.) in the Free State is also to be determined; specifically, the regions of origin of the current giraffe population in the region should be established. From this study, researchers will gain knowledge on the effects of isolation and fragmentation on the giraffe populations that can ultimately be used to guide management and legislative / policy decisions by private and public conservation managers.

1.1 General giraffe biology

1.1.1 Evolution of the giraffe

Spinage (1968) asserts from primitive rock paintings found scattered in Africa, that giraffe roamed Northern Africa until 500 B.C. It is assumed that after the desertification of northern Africa occurred, the species migrated southwards to more suitable habitat. It is generally accepted that the pre-desert Sahara region consisted of lush vegetation with numerous river systems (Spinage 1968). Rock paintings of giraffe-like animals have been found in many northern African and even European countries. The ancient Greeks referred to the giraffe as “Camelopardalis” due to the apparent dual nature of this ungulate. By dual nature, the Greeks referred to the physical appearance of the giraffe, whereby it was thought that a giraffe was a camel with the spots of a leopard (Spinage 1968).

The evolution of giraffe has been the subject of considerable debate. According to Lonnig (2011), statements on giraffe evolution have been made in the past without the backing of substantial evidence. This is mostly seen in the flawed theories concerning the evolution of the modern-day giraffe from the original ancestral animal the giraffe evolved from (Lonnig 2011).

The different theories proposed to account for the unique morphology of the giraffe are well-known in the field of evolutionary biology. In particular, Jean-Baptiste Lamarck and Charles Darwin proposed contrasting theories. Lamarck suggested that there was constant change in an ever-changing environment (Holdrege 2003). This

early scientist concluded that animals could change depending on the environmental conditions and the need to survive, i.e. giraffe had to keep stretching their necks in order to reach food at high levels in the trees, and the neck became longer from generation to generation (Pun 1982). Darwin, however, believed that when there were particular environmental conditions, specific variations within organism populations were advantageous. This theory is based on the use of underlying genetic diversity through the process of natural selection. Based on Darwin's ideas, mal-adapted organisms would die off, whereas the best-adapted organisms would survive to pass on the advantageous genes (Holdrege 2003; Kampourakis and Zogza 2007). Today, Darwin's theory (Figure 1.1b) is accepted by scientists whereas Lamarck's theory (Figure 1.1a) regarding the development of the giraffe's neck has been largely discredited.

The elongated neck of the giraffe has various advantages including dissipation of heat and establishing social hierarchy (Hughes 1979; Cameron and du Toit 2007). It has also been suggested that the long neck confers an advantage over other browsers, in reducing competition for food by facilitating browsing at levels of trees not utilized by other grazers (Cameron and du Toit 2007). This theory has, however, been disputed by Lonnig (2011), who stated that seasonal food availability also plays a role.

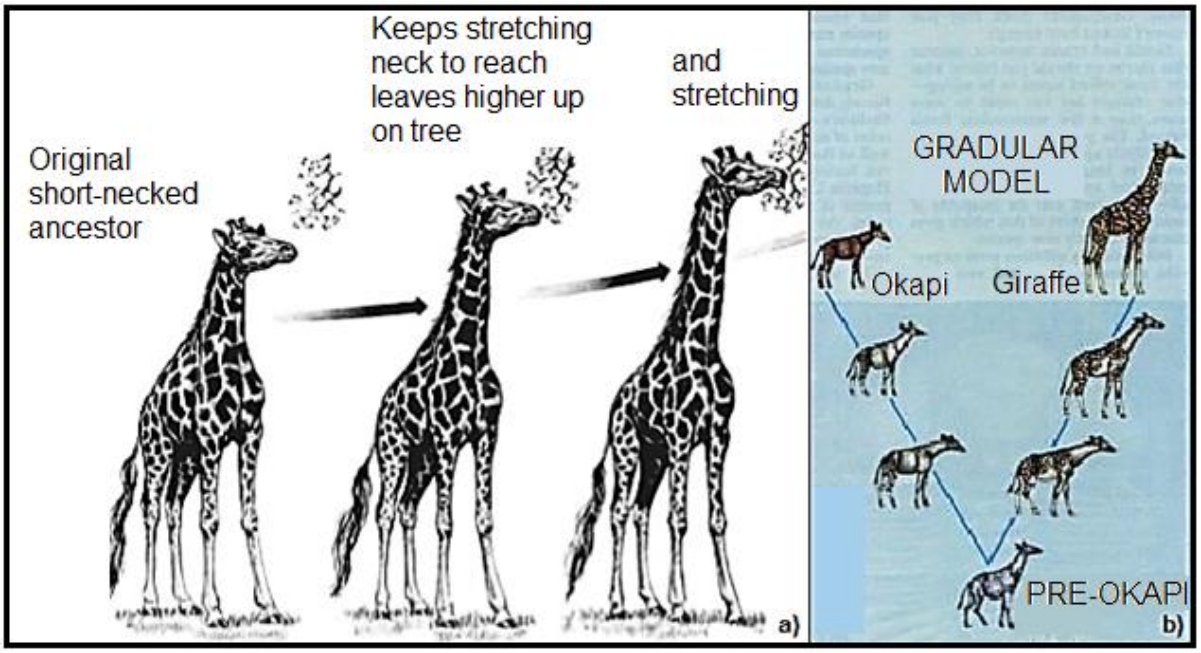

Figure 1.1 A comparison of evolutionary theories by a) Lamarck and b) Darwin taken from Pun (1982) and Allen (2012).

1.1.2 Morphology and biology of the giraffe

The giraffe (*Giraffa camelopardalis* Linnaeus 1758) is one of the older ungulate species found (Spinage 1968; Grzimek 1972; Hughes 1979; Simmons and Scheepers 1996; Mitchell and Skinner 2003; Fennessy 2004), descending from a primitive deer-like mammal which originated from the Palaearctic region (Hughes 1979). Grzimek (1972) suggested that giraffe seemed to have evolved about 25 million years ago. According to Crandall (1964), the extant family Giraffidae consists of two genera, namely *Giraffa* and *Okapia*. These genera have several shared physical characteristics. Colbert (1935) described how the derivation of the Giraffidae family came about, showing the rapid evolution of the subfamilies and genera. Some of the shared characteristics include the elongated neck which is more exaggerated in the giraffe, and skin-covered “horns” (called ossicones) that are found on both male and female giraffe, but are present on male okapis only (Crandall 1964).

The main morphological differences between the ancestral species and the modern-day giraffe are shown in Figure 1.2. Characteristics of the primitive giraffe ancestors included a short neck and being of medium size, while also still possessing many deer-like features (Grzimek 1972). As evolution progressed through natural selection, the modern-day giraffe acquired unique and/or adaptive characteristics. This included the size of the animal changing through evolutionary time from medium-sized to large or very large, the neck being elongated, longer legs with the front pair being longer than the hind pair, and still possessing a pair of structures (ossicones) on their foreheads which are covered in skin, although slightly smaller than before (Dagg 1971; Grzimek 1972; Apfelbach 1990).

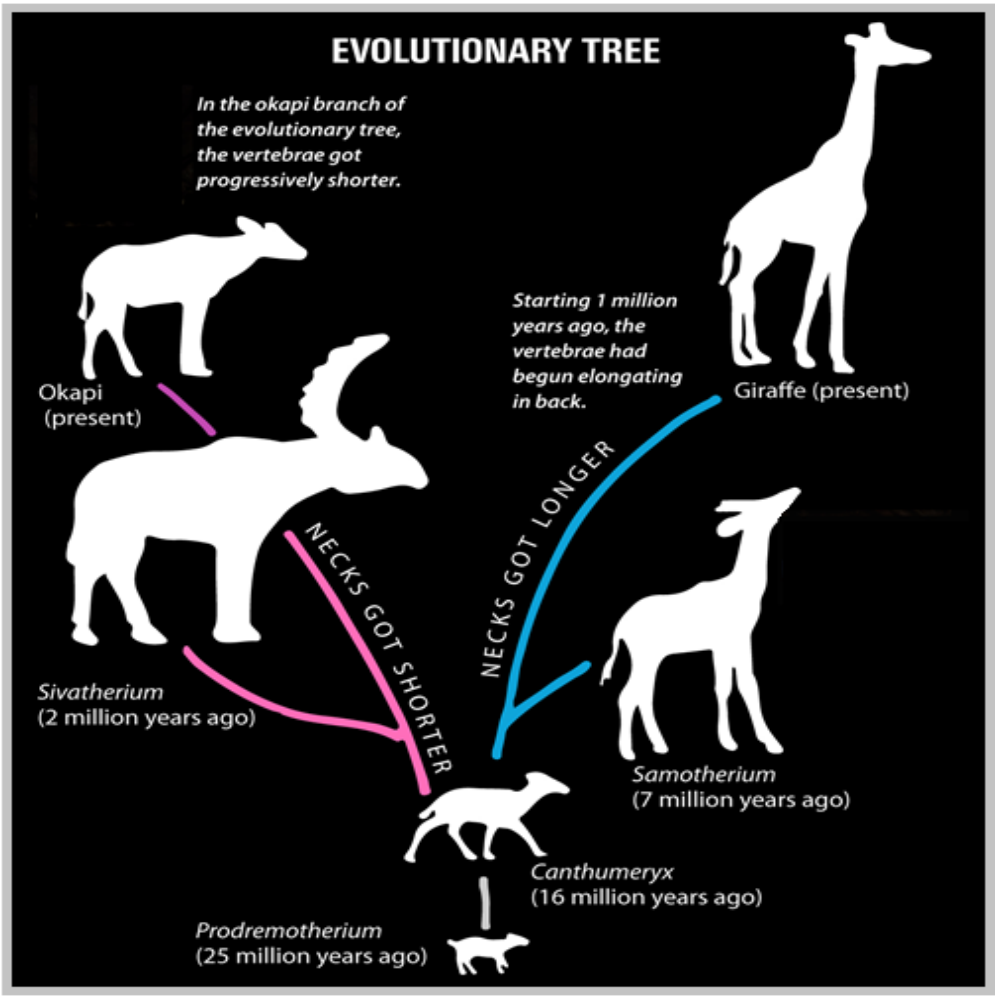

Figure 1.2 Evolutionary tree showing the ancestral species which ultimately diverged to form the two existing genera of the family Giraffidae (Illustration from Danowitz *et al*. 2015).

Giraffe are classified as the tallest animals on earth, with a height ranging from 5-6m (Dagg 1971). Weight ranges from 500-750 kg (Grzimek 1972). Giraffe have elongated faces, with comparatively big eyes, short pointed ears, a narrow snout, and a pair of skin-covered horns on the forehead called the ossicones. Depending on the subspecies, giraffe have between two and five bony knobs situated just below their horns (Apfelbach 1990). There is a difference, however, in the horn appearance between the sexes; although the horns of both sexes are almost entirely covered in skin, those of females have hair at the tips, whereas the horns of males are without hair at the tips (Grizmek 1972).

Giraffe are gregarious animals that are commonly found feeding on browsable material alongside other wildlife species, including wildebeest (*Connochaetes sp.*) and

zebra (*Equus quagga*), as well as other antelope species and ostriches (*Struthio camelus)*. There have been many theories as to why this association may occur. Foster (1966) has suggested that an advantage from this type of gregarious behaviour is that giraffe have a better outlook of the habitat from a viewpoint perspective. Giraffe can thus spot danger much easier than other animals, thereby alerting them, increasing the potential of the other species to survive. Giraffe thus co-exist well with the other ungulate species, due to the fact that there are security benefits for all species involved (Lockwood 2015).

The unique feeding habits of the giraffe confers specific advantages and disadvantages. The feeding benefits from feeding at high levels, and partitioning of the tree utilisation by different browsers, was described by Cameron and du Toit (2007) and Lockwood (2015). In this regard, steenbok (*Raphicerus campestris*) and impala (*Aepyceros melampus*) browse from the lowest levels possible, greater kudu (*Tragelaphus strepsiceros*) and eland (*Tragelaphus oryx*) eat from higher levels, and giraffe eat from the highest possible levels. Both Du Toit (1990) and Fleming *et al.* (2006) described how the flowers of the African knobthorn (*Acacia nigrescens*) was a beneficial food resource in dry seasons. These authors also explained that giraffe need large quantities of food to sustain themselves, and to achieve this, they tend to eat large amounts of food to compensate for low nutritional value.

1.1.3 Giraffe taxonomy

Giraffe taxonomy and subspecies status have been the subject of much debate over the last century (Lydekker 1904; Colbert 1935; Singer and Bone 1960; Dagg 1962; Sidney 1965; Dagg 1971; Hughes 1979; Lonnig 2011). With the translocation of giraffe occurring more frequently in recent times, without knowledge of their genetic background, natural patterns of diversity and differentiation may potentially be disturbed due to the mixing of distinct taxonomical units.

Bercovitch and Deacon (2015) stated that there are currently at least four different taxonomic classifications for giraffe. Each classification system has a different number of species and subspecies. Fennessy *et al*. (2016) recently suggested a new taxonomy for giraffe, using multi-locus analyses to reveal that there are four genetically

distinct giraffe species, instead of one. In the study by Fennessy *et al*. (2016), nuclear DNA data was analysed from the 'formerly' recognised giraffe subspecies. These authors state that the new findings regarding the taxonomy of the giraffe will have conservation implications, as giraffe numbers keep declining due to human-induced threats. The subspecies found in South Africa, according to Bradford (2014) is *Giraffa camelopardalis giraffa* von Schreber, (1784). This South African giraffe subspecies is one of the nine subspecies recognised by Dagg (1971); Ansell (1972); Dagg and Foster (1982); Kingdon (1997); East (1999); Grubb (2005); Ciofolo and Pendu (2013); Deacon and Parker (2016). There have been multiple studies conducted on using skin patterns and the morphology of the giraffe to aid classification (Colbert 1935, Singer and Bone 1960, Dagg 1962, Crandall 1964, Grzimek 1972, van der Jeugd and Prins 2000, Giraffe Conservation Foundation 2015). Morphology-based classification have differed significantly between various researchers (Bercovitch and Deacon 2015). Suggested taxonomy varies, as seen in Table 1.1.

Table 1.1 A list of various suggested taxonomy of the giraffe.

Author	Year	Number of Species	Number of Subspecies
Lydekker	1904	2	10
Dagg and Foster	1976	1	9
Kingdon	1997	1	8
East	1999	1	6
Grubb	2005	1	5
Fennessy *et al*.	2016	4	5

With the general decline in giraffe numbers, the IUCN (Fennessy and Brown 2010) listed two subspecies as "Endangered" and determined the broader giraffe classification as "Vulnerable" (Muller *et al*. 2016). However, the South African subspecies is listed as of "Least Concern". It is estimated by Deacon and Tutchings (2018) that there are between 21 503 – 26 919 giraffe individuals living in South Africa, but an accurate estimate of the number of individuals is difficult to obtain. These authors claim that there are a large number of translocations of giraffe occurring in South Africa, since this species can be privately owned. Nature Reserves, as well as established privately owned wildlife farms, are also translocating new breeding males from one population to another, with the aim of introducing new genetic material into the population, thereby increasing the genetic diversity of these populations.

Other than geographical separation, each subspecies displays differences in skin patterns (Colbert 1935, Singer and Bone 1960, van der Jeugd and Prins 2000, Giraffe Conservation Foundation 2015), in the number of ossicones (horns) found on the heads of the various giraffe populations (Dagg 1962, Crandall 1964, Grzimek 1972), as well as differences in mitochondrial haplotypes (Brown *et al*. 2007, Brenneman *et al*. 2009a), making them genetically unique.

1.1.4 Distribution of the giraffe

1.1.4.1 Natural distribution patterns of giraffe in Africa

Giraffe originated from the African savannah plains (Apfelbach 1990), and over a period of time dispersed to other geographic locations south of the Sahara Desert. This pattern of dispersal was confirmed by Grzimek (1972), who stated that fossils found in North Africa suggested a northern African origin of the giraffe. McCarthy (2008) suggests that giraffe were once very diverse and were found over a wide geographical distribution. It has been implied that giraffe dispersed southwards as northern Africa became drier, ultimately leading to the disappearance of the giraffe from northern Africa (Dagg 1971). The localities of where the nine currently recognized subspecies can be found in Africa are shown in Figure 1.3.

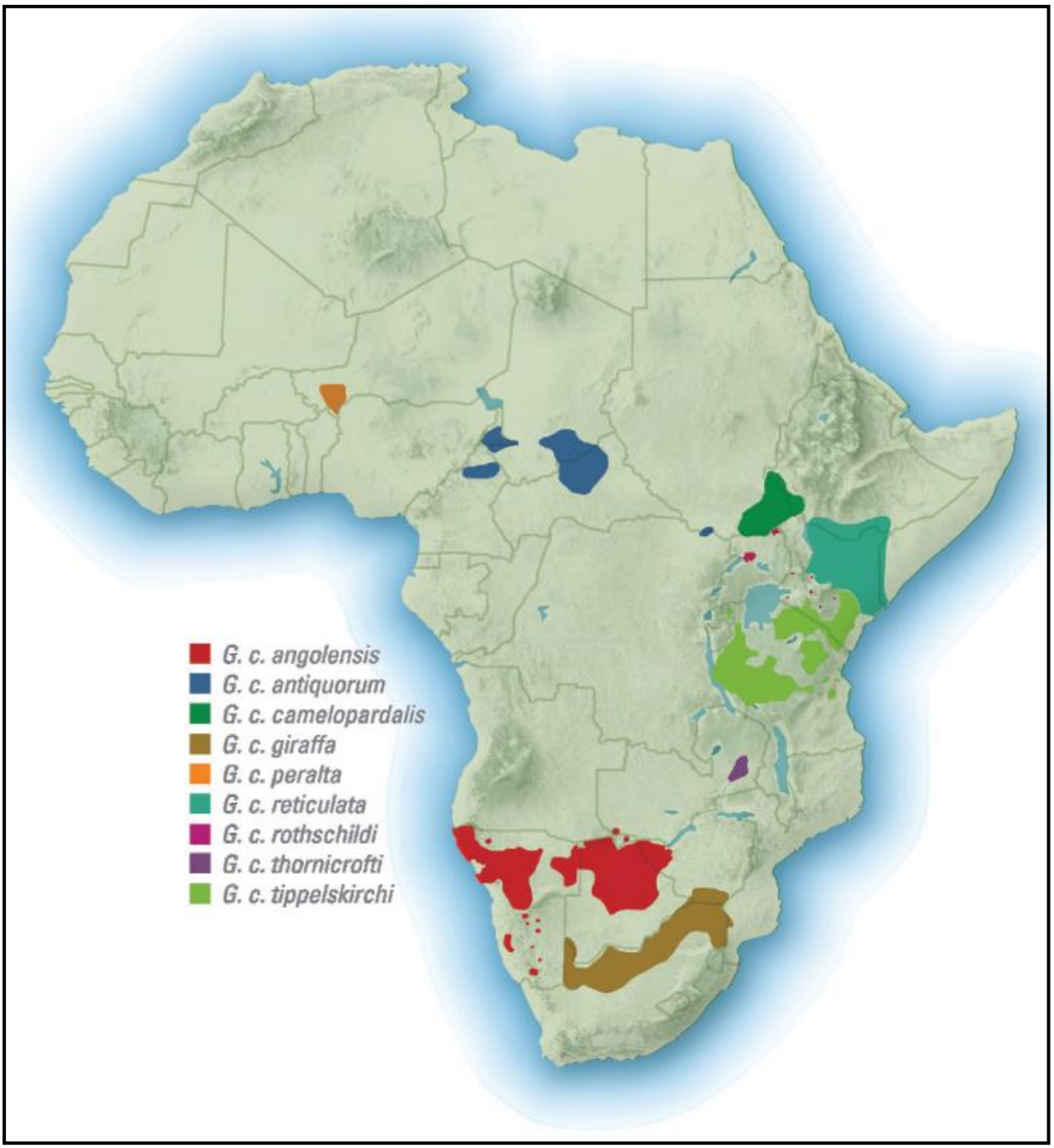

Figure 1.3 A distribution map of the localities of all 9 subspecies of *Giraffa camelopardalis* compiled by the Giraffe Conservation Foundation (2015).

1.1.4.2 Natural distribution patterns of giraffe in South Africa

The natural distribution range of giraffe in South Africa, both historically and presently, is described as uncertain by Deacon (2015). There are various reasons for this. According to this author, giraffe location records originally showed a distribution range extending from the Limpopo Province, to KwaZulu-Natal and the North West Province. With an increase in the number of game farms that keep giraffe in recent times, the current distribution range of *Giraffa camelopardalis giraffa* has effectively extended to other Provinces that previously did not harbour giraffe. This resulted in a significant number of extralimital populations, with giraffe introduced to areas where they did not occur historically. Deacon and Tutchings (2018) published data pertaining to the natural distribution of giraffe in South Africa (Figure 1.4). The distribution map was created by using data which dated up to 500 years ago, as well as habitat suitability based on vegetation types.

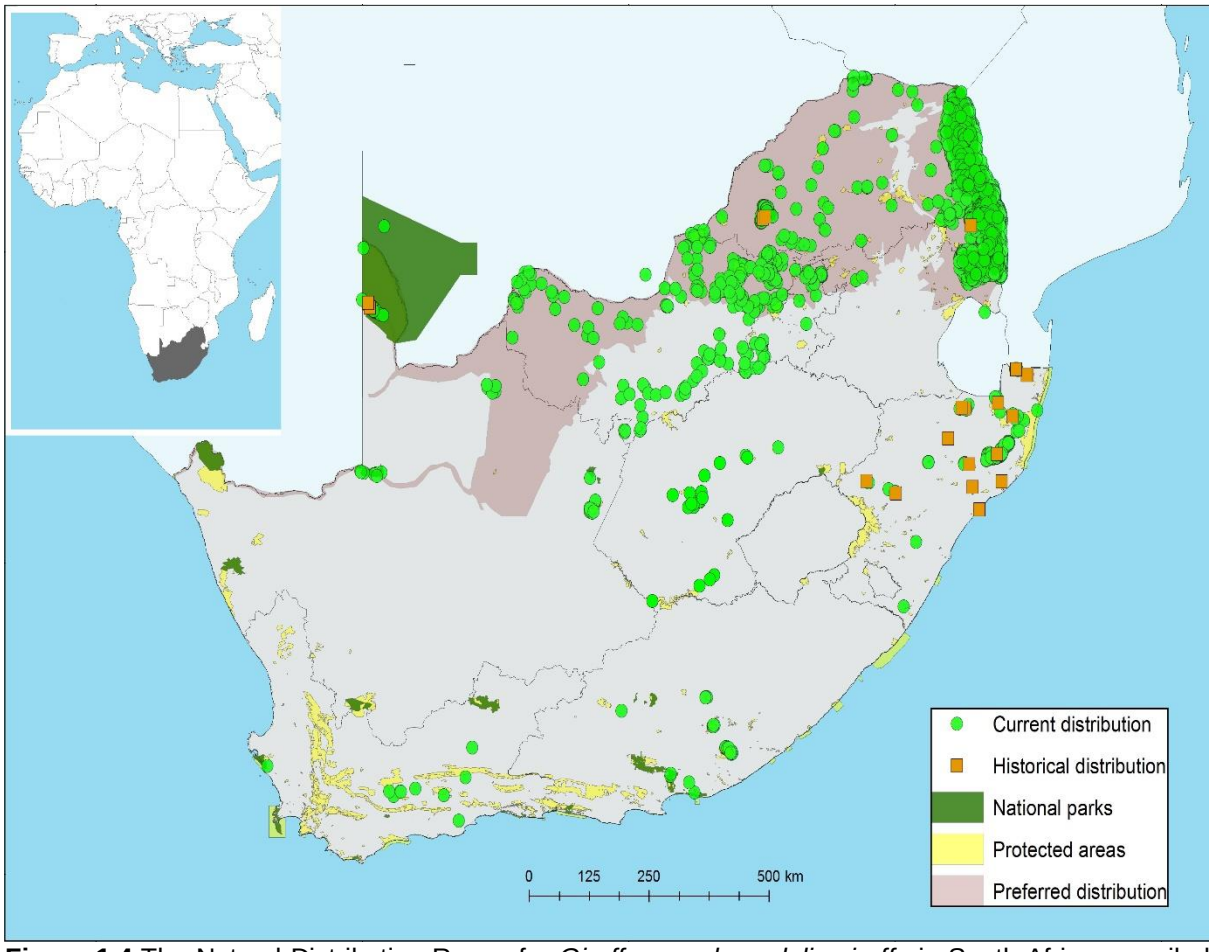

Figure 1.4 The Natural Distribution Range for *Giraffa camelopardalis giraffa* in South Africa compiled by Deacon and Tutchings (2018).

1.1.5 Modern distribution of giraffe in the Free State Province, South Africa

1.1.5.1 Current distribution of giraffe in the Free State Province

Giraffe were not originally found within the Free State Province, thus making them extralimital to the province. The South African Department of Environmental Affairs has published information which verifies the previous statement [Department of Environmental Affairs, 2018]. There is no comprehensive information to verify at the current stage, exactly how many giraffe there are on private properties in the Free State, however, the exact number of individuals within Nature Reserves within the Free State is monitored by the Free State Department of Economic, Small Business Development, Tourism and Environmental Affairs (DESTEA) in 2016.

1.1.5.2 Current distribution of giraffe within the different Free State Nature Reserves

The Free State Province is divided into five regions, namely Lejweleputswa, Mangaung, Fezile Dabi, Thabo Mofutsanyana and Xhariep (Lehohla 2011). There are 14 Provincial Nature Reserves in the Free State (Figure 1.5), with 1 National Park; and many privately-owned farms, reserves and ranches[1]. Of the 14 Provincial Nature Reserves in the Free State Province, only three have giraffe populations.

Information obtained from DESTEA indicates the translocation of giraffe between various nature reserves[2], especially two within the Free State, namely Sandveld Nature Reserve and Willem Pretorius Nature Reserve. Ulysses SA (2016) mentioned that Willem Pretorius Nature Reserve is the oldest Nature Reserve in the Free State, covering about 12 000ha, where Sandveld Nature Reserve in the Western Free State covers 37 000ha. Figure 1.6 shows the translocation of giraffe from three localities to Willem Pretorius Nature Reserve between 1963 and 2003. The translocation of giraffe into Sandveld Nature Reserve from 3 localities, including Willem Pretorius Nature Reserve is also shown. Finally, translocations of giraffe from

[1] Personal communication with Erika Schulze, Free State Department of Economic, Small Business Development, Tourism and Environmental Affairs, Free State Province, South Africa.
[2] Personal communication with Pierre Nel, Veterinarian, Free State Department of Economic, Small Business Development, Tourism and Environmental Affairs, Free State Province, South Africa.

both Willem Pretorius Nature Reserve and Sandveld Nature Reserve to other localities are also indicated in Figure 1.6.

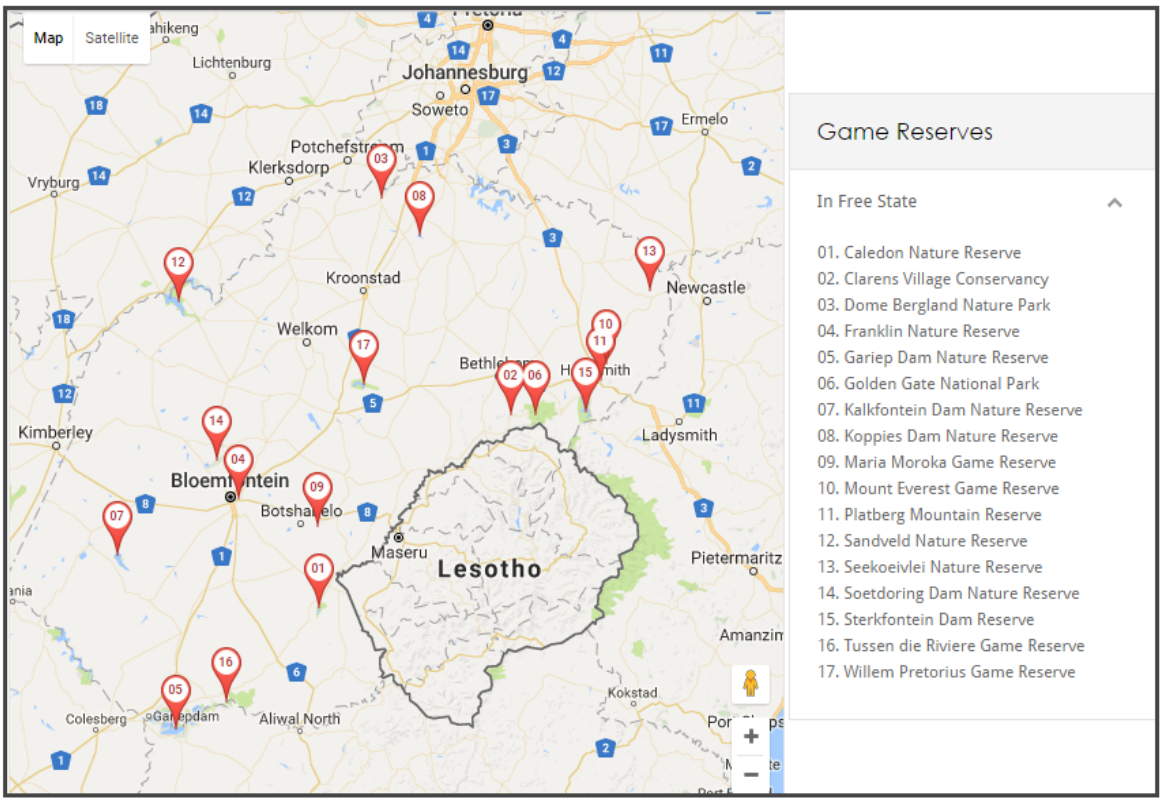

Figure 1.5 Map of Provincial Nature Reserves in the Free State, South Africa taken from Anonymous (2016).

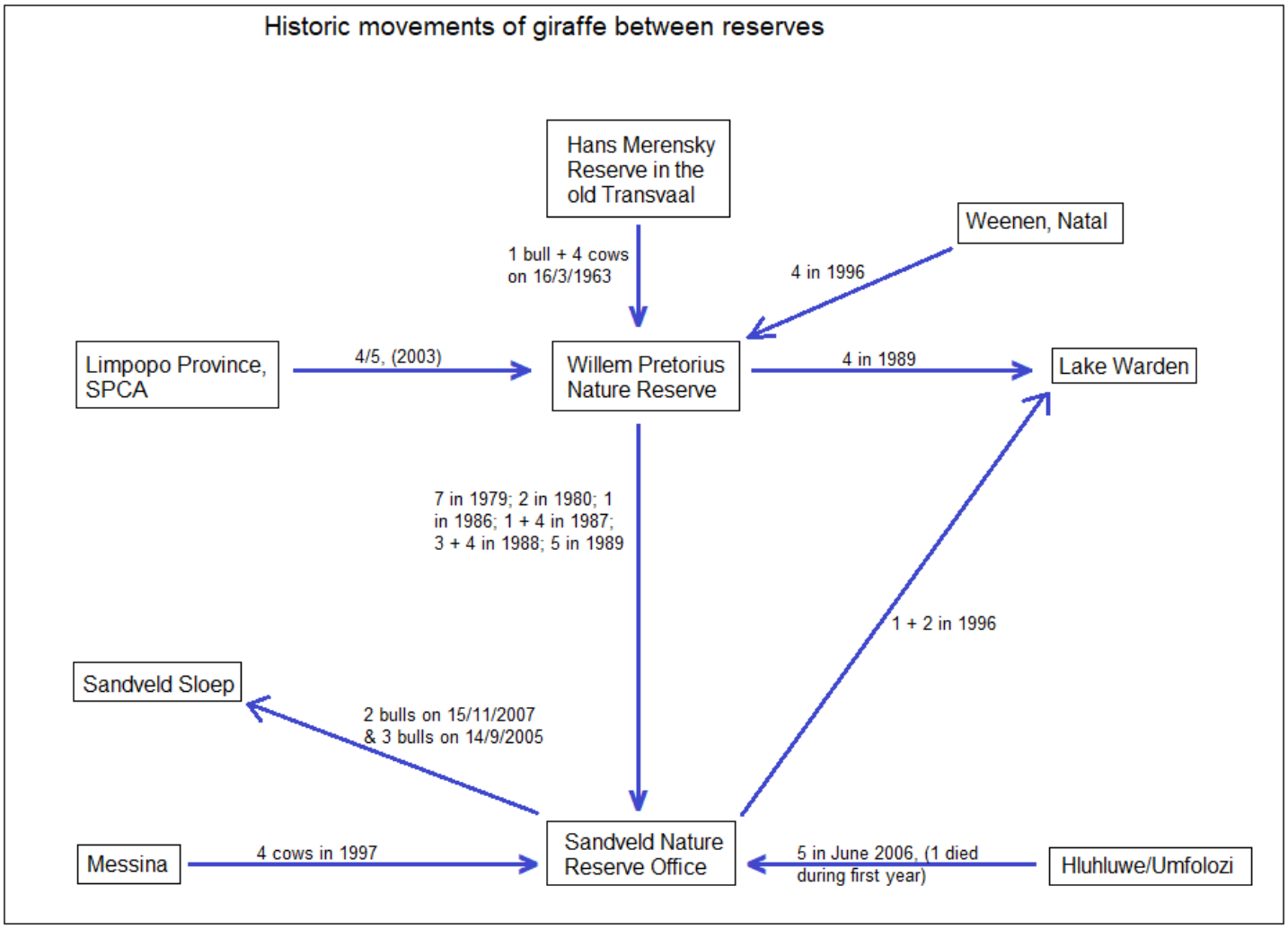

Figure 1.6 The historic movement of giraffe between Free State reserves. Information supplied by DESTEA.

From a genetic perspective, the fact that there have been translocations from various localities means that there is potentially more genetic diversity within such populations, thereby decreasing the likelihood of inbreeding (Lacy 1987). Conversely, the founding of some populations with insufficient sizes, followed by a lack of gene flow, may result in the loss of genetic diversity within populations and an increase in inbreeding (Furlan *et al.* 2012)

1.1.5.3 Current distribution of giraffe on private land in the Free State Province

Giraffe were not historically found in the Free State (Deacon and Parker 2016). However, there are currently many privately-owned farms, reserves and ranches in the Free State which have giraffe despite the historical absence of the species from the Province (Deacon and Parker 2016), resulting populations that can be classified as extralimital. Limited information is available on numbers and occurrence of giraffe on privately-owned farms. In contrast, DESTEA conducts frequent censuses of the animals and therefore have comparatively accurate records on numbers of giraffe on Provincial reserves. The number of giraffe found on the farms range from a single giraffe to sizable populations. Giraffe are, however, almost invariably found in small populations, for reasons of habitat availability or because they may not be as prized on private farms as some other species.

1.2 Fragmentation of populations

From a genetic diversity perspective, confinement or isolation of a population causes a risk of decreased genetic diversity, and fragmentation could possibly increase the occurrence of inbreeding in the population (Klug *et al*. 2009). Fragmentation increases genetic drift, as well as leading to a higher risk of the species or local populations ultimately becoming extinct (Dixo *et al*. 2009).

Confinement on game farms and small reserves, coupled with a lack of gene flow with the wider national giraffe population, can result in local loss of genetic diversity. This could potentially increase the occurrence of inbreeding in populations.

The negative effects of inbreeding are well-known. In the short term, this can result in reduced fitness, with potential effects such as increased mortalities and reduced resistance against unfavourable condition (Ralls *et al*., 1979; Hedrick, 2000). In the longer term, loss of genetic diversity limits the ability of populations to adapt to changing environmental circumstances.

Conversely, the translocation of an individual from an isolated area to another area, could also cause disruption to the genetic make-up of the group if taxa are sufficiently different and if breeding should occur (Scribner 1993). The occurrence of such outbreeding in populations could potentially result in reduced fitness (Edmands 2006). Though giraffe are generally assumed to be fairly homogeneous across their distribution in South Africa (Spear and Chown 2009), there have been some reports of translocations from further afield. Mixing of genetic variants adapted to local conditions with imported animals can result in (i) unwanted mixing of distinct taxonomic or ecological units (Crandell *et al*. 2000, Moritz 2002); and (ii) fitness effects known as outbreeding depression (Edmands 2006). The latter can, for example, lead to the disruption of the timing of biological events (e.g. calving season).

Conservation geneticists have different levels of interest. The levels are described by Woodruff (2001) as a) genes; b) populations; c) subspecies; d) species; and e) communities. According to Bercovitch *et al*. (2017), taxonomic interpretations should be based on more than one factor, namely on species, morphology, population distribution, ecology, and behaviour. Loew (2002) stated that the major levels of interest are to minimize the loss of genetic diversity within populations, as well as defining taxonomic units which should be conserved. The aims of the current study were formulated with this guideline in mind.

Isbell (2010) stated that the decline in many wildlife populations is due to the effects of global warming, habitat fragmentation, habitat loss, and over-exploitation. According to this author, habitat fragmentation increases the distances between habitat patches that are occupied by populations, which causes population connectivity to be reduced (Mora *et al*. 2007). From a South African perspective, the existence of numerous fences plays a similar role. However, the introduction of new individuals within a population ensures the introduction of new alleles, resulting from increasing gene flow and reducing inbreeding within the population (Klug *et al*. 2009).

Mora *et al*. (2007) further discuss how overexploitation also causes damage to a population, ultimately causing the genetic diversity of that population to decrease. While the decline in genetic diversity could occur in any population, there is a greater risk for smaller populations. Small populations have a greater chance of reduced genetic diversity, and an increased chance of inbreeding, ultimately leading to the population becoming extinct (Loew 2002). Brown *et al*. (2007) stated that due to the severe poaching and armed conflict in Somalia, Ethiopia, and Kenya, the number of reticulated giraffes was reduced from 27 000 individuals in the 1990's, to less than 3000 individuals.

The effects of inbreeding can be fatal, where reproduction performance of naturally outbreeding populations can be reduced, thereby possibly reducing terms of survival (Allendorf and Luikart 2007). Inbreeding tends to produce less fit offspring than offspring produced by random mating. The management of populations should thus aim to increase levels of genetic diversity, thereby increasing the individual's fitness within the population, and allow for future evolutionary adaptations to occur (Klug *et al*. 2009). Loew (2002) stated that as the size of a population decreases, mortality rates tend to increase, thereby affecting the gene frequency and gene flow within the population.

Deacon (2015) explained how fragmentation of giraffe populations within South Africa has secluded many South African giraffe populations. These authors noted that a large proportion of giraffe in South Africa have been translocated from National Parks and Provincial Nature Reserves, and translocations could possibly result in the hybridization of the subspecies. This is due to the fact that not much is known about the taxonomic status of giraffe in the various provinces, notably the Free State Province.

1.2.1 Mechanisms of fragmentation

1.2.1.1 Reasons for the occurrence of fragmentation

When studying fragmentation, individuals within a population and how the individuals interact with one another should be considered. It is important to consider that individuals can migrate from one population to another, thereby moving from a possibly favourable habitat to an unfavourable one and altering population size. Lastly, the entire geographic range of the species should be taken into consideration (Allendorf and Luikart 2007).

The habitat in which giraffe live is under constant threat. According to Fynn and Bonyongo (2010), there is a continual decline in the size and diversity of ungulate populations which are found in conservation areas in Africa. If the habitat decreases, the distribution area of the species is affected negatively. The fragmentation of habitat can also drive a population to become isolated. By limiting a species to a confined area, problems would start to occur in relation to the genetic make-up of the population. A phenomenon that has gone unnoticed by many stakeholders is the decline in the number of giraffe, as evident from the few articles that have been written on the situation (Hughes 1979, Fynn and Bonyongo 2010, Smitz *et al*. 2014, Giraffe Conservation Foundation 2015). A decline of approximately 40% of the giraffe population in Africa over the past 15 years, has left only 80 000 individuals remaining across the nine subspecies (Tutchings 2014).

Fragmentation of populations are being driven by many factors, one being the increase in human population densities (Dagg 1962). If human settlements increase, conservational areas holding valuable species decrease. Currently, many animals also come under threat due to poaching and the interference from humans in protected areas (Dagg 1971, Hughes 1979). Habitat loss and vegetation fragmentation are however regarded as the biggest factors and reasons for the decline in giraffe across Africa (Deacon *et al*. 2016).

As stated by Deacon and Parker (2016), the game ranching industry in South Africa is adding value to the overall giraffe population by increasing numbers. In contrast to the general population decline of giraffe in Africa, the number of giraffe on private game ranches in South Africa are increasing. However, there are problems related to fragmentation. Farmers buy giraffe for aesthetic value, however, some do

not consider the fact that by translocating giraffe, they are moved to habitats for which they are not optimally adapted.

1.2.1.2 The consequences of fragmentation on populations

Scribner (1993) states that human activities are increasingly impacting natural populations of species. This includes uncontrolled sport hunting, poaching, translocation, as well as human impacts on the environment causing fragmentation. These particular factors can have a detrimental effect on animals, including the demographics of the population (age, sex, and size), how the species breeds, as well as the levels of genetic variation (Scribner 1993). In severe cases, fragmentation could even result in localised extinctions (Wilcox 1980). Described below are a few cases where fragmentation has had an impact on specific populations.

1.2.2 Case studies of fragmentation in various mammal populations

1.2.2.1 Fragmentation occurring in ungulate populations

Fragmentation can equally affect private and public animal populations. Private fragmented populations can be found in the wildlife ranching sector, whereas public fragmented populations refers to the populations in National Parks and game reserves owned by the Government. The reasons for fragmentation in both populations are often similar, and the genetic effects can also be very similar.

Wildlife ranching is being done on a large scale in South Africa, with an estimated 9 000 wildlife properties (*Taylor et al*. 2016). Cousins *et al*. (2008) mentioned that several challenges arise because of the small, enclosed character of many ranches in South Africa, including the need to intensively manage wildlife populations. Government owned protected areas, including National Parks and game reserves, cover 5% of the total land area, where privately-owned wildlife ranches in South Africa cover 16.8% of the total land area (Cousins *et al*. 2008). In an article by *Taylor et al*. (2016), it is noted that in the 1980's there were very few game reserves in South Africa outside the traditional areas linked to the Kruger National Park, however, the number of game reserves has increased at a rapid rate.

Smitz *et al*. (2014) noted that there has been a decline in population size and geographical distribution of African wildlife, due to several factors, such as the increase in human demography, wildlife overexploitation, habitat degradation, as well as the increase in diseases. It is stated that ungulate populations are largely confined within a network of loosely connected protected areas (Smitz *et al*. 2014). Davies-Mostert *et al*. (2009) mentioned that many species have been reduced to small, fragmented populations. McNaughton and Georgiadis (1986) state that mammalian fauna has become increasingly isolated and fragmented within game reserves.

1.2.2.1.1 Fragmentation occurring in rhino populations

Rhinos provide an example of the effect of recent anthropogenic fragmentation. As the total number of rhinos decreases rapidly and the world faces the total extinction of all rhinos, conservationists are trying to find possible solutions to curb this risk. Predictions at that time were that by the year 2020, all rhino species would be extinct (Ahmed 2014). According to the World Wildlife Fund (2016), a total of 1 175 rhinos were killed in South Africa in 2015, compared to the 1 215 killed in South Africa 2014. The problems surrounding rhino populations affect both species found in southern Africa.

The African black rhinoceros (*Diceros bicornis*) has seen one the most drastic declines in population size due to intensive poaching and habitat fragmentation (Karsten *et al*. 2011). The black rhino consists of four recognised subspecies (Emslie 2012b), namely *Diceros bicornis bicornis*; *Diceros bicornis longipes*; *Diceros bicornis michaeli*; and *Diceros bicornis minor*. The species as a whole is classified as Critically Endangered (Emslie 2012b). Harley *et al*. (2005) indicated that between 1970 and 1992, 96% of the population had been eradicated, leaving only a few widely spaced population fragments. There were only 3 610 individuals remaining in 2005, with 49.17% of these individuals located in South Africa (Harley *et al*. 2005). With the distribution of this species now limited to isolated populations in a few countries, the survival of the species is seriously threatened.

The white rhinoceros (*Ceratotherium simum*) is also undergoing a decline in numbers, for the same reason as the African black rhinoceros. For this species there

are two recognised subspecies (Emslie 2012a), namely *C. s. simum* and *C. s. cottoni*. The species as a whole is classified as Near Threatened (Emslie 2012a). The World Wide Fund for Nature (WWF 2017) stated that white rhinos were thought to be extinct in the late 19th century. By 1985, a population of less than 100 individuals occurred in KwaZulu-Natal, South Africa. After the successful protection and management of the species, the number of individuals increased to approximately 20 000 individuals. Even though white rhino are not one of the endangered rhino species, this species has unfortunately suffered the pressure of being the most poached (WWF 2017).

1.2.2.1.2 Fragmentation occurring in wildebeest (*Connochaetes taurinus*) populations

One of the most well-documented wildlife phenomena that occurs in East Africa is the annual wildebeest migration in the Serengeti. During this period, millions of wildebeest and other ungulates migrate between the Serengeti and Masai Mara ecosystems (in Tanzania and Kenya respectively), in search of water and a habitat with enough food supply (Sinclair and Arcese 1995). The different habitats play an important role in the different seasons. For example, the short grass in the southern Serengeti provides valuable high protein for the ungulates during the wet season, whereas the moderately nutritious taller grasslands in northern Serengeti provides last resort resources during the dry season (Owen-Smith 2004). The Tanzanian Government recently announced plans to build a commercial highway through the Serengeti National Park, which would ultimately divide the park into two (Holdo *et al*. 2011). The construction would affect the annual migration of the ungulates and would cause collapse due to the fragmentation of the natural migratory patterns (Friends of Serengeti 2014). The impact caused by the highway could be catastrophic. Not only would the migration be affected, poaching could likely increase, along with levels of pollution caused by the vehicles using the road, and the predicted increase in possible collisions with animals. Within the migratory routes are three habitat types, namely thorn savanna in the central and western sections of the park, dry plains in the south-east, and moister mixed woodlands in hilly terrains in the north (Owen-Smith 2004).

The reasons for fragmentation occurring in other ungulate populations are also applicable for giraffe populations. Fragmentation of natural migratory patterns due to

the construction of roads through National Parks, as well as poaching and other human interferences also contribute to the fragmentation occurring in giraffe populations (Dagg 1971, Hughes 1979, Holdo *et al*. 2011). South Africa has a history of ungulate introductions and extralimital translocations (Spear and Chown, 2009). Translocations, small populations, limited habitat availability, and small enclosures all influence ungulates in South Africa.

1.2.2.2 The occurrence of fragmentation in giraffe populations

Giraffe were once distributed throughout Africa, from the northern African countries to the most southern African country (Brown *et al*. 2007). However, due to the increase in aridity in northern Africa over a period of time, as well as population growth, the current geographic range of giraffe has been severely reduced (Brown *et al*. 2007).

As described by the Wildlife Conservation Society (2015), the unique and isolated Thornicroft's giraffe (*Giraffa camelopardalis thornicrofti*) is only found in Zambia's South Luangwa Valley and there are currently less than 1 000 individuals of this particular subspecies remaining (Giraffe Conservation Foundation 2015). In Uganda, Rothschild giraffe (*Giraffa camelopardalis rothschildi*) is considered endangered (Fennessy and Brenneman 2010, Giraffe Conservation Foundation 2015). In both countries, conservationists are attempting to educate local communities about the importance of the species and are trying to teach people how to protect the habitat in which they live (Giraffe Conservation Foundation 2015). By establishing Conservation parks, stakeholders attempt to encourage the conservation of the species.

Following the work of Berry (1978) on the *G. c. thornicrofti* population in the South Luangwa Valley, 27 giraffe (16 bulls, eight cows, and three juveniles of undetermined sex) were found in an area around the Luangwa River, consisting of twice the number of males compared to females. With little migration occurring to and from the western side of the river, Berry (1978) found that continued reproduction is a cause for concern due to the small number of females in the population. In contrast, the giraffe population on the eastern side of the river has a larger population size. Movement of males was suggested to be determined by the location of the females,

however, it was not uncommon to find some solitary males roaming around the area. Berry (1973) stated that the number of individuals in the giraffe population increased slightly between 1964 and 1969; however, it was also stated that the accuracy of the estimated number of individuals was questionable due to the loose herd association which changed often.

There are less than 1 100 Rothschild's giraffe remaining in the wild, with most being in Uganda, and with a few isolated populations in Kenya (Tutchings 2014). Brenneman *et al*. (2009b) found that there was only one population remaining in Uganda and four populations in different Parks and Reserves in Kenya at the time. Awange *et al*. (2004) explained that between 1970 and 1980, this particular subspecies was relocated from a ranch in the Kenyan Rift Valley to two National Parks in Kenya, namely Ruma National Park and Lake Nakuru National Park. The number of individuals in the Lake Nakuru National Park decreased dramatically between 1995 and 2002, from 153 individuals to a mere 62 individuals (Brenneman *et al*. 2009b). The decrease in the number of individuals was attributed to the extreme climatic conditions resulting from the 1994 El Nino effect. Brenneman *et al*. (2009b) stated that due to the drought that occurred, there was a decline in the number of acacia trees in the park, which made many young giraffe easy prey for predators due to them being weak and vulnerable.

Fennessy *et al*. (2016) suggested that South Africa harbours only the South African giraffe. Deacon and Parker (2016) states that the South African giraffe has been translocated to other countries from South Africa and, significantly, the Angolan giraffe has been translocated from Namibia to countries such as Botswana and South Africa. Deacon and Parker (2016) also suggest that the total number of South African giraffe are increasing in total, even though populations are severely fragmented in all areas, in addition the transfrontier parks.

1.3 Geographic genetic structure

The inconsistent gene flow among offspring is the reason why there is differentiation in subspecies, which are widely spread across a geographical distribution, ultimately causing speciation (Klug *et al*. 2009). Factors influencing the structuring of a population could include landscape features (Coulon *et al*. 2006), isolation of a population, and differentiation within a population due to migration and translocation (Wright 1949). Wright (1949) stated, however, that isolation without marked environmental differences, present the most favourable conditions for transformation of a species.

Brown *et al*. (2007) performed a study on the population genetic structure in the giraffe from six localities across Africa. These authors suggested that there are nine subspecies of giraffe, based on morphology. However, the differences in the skin patterns of the various subspecies are not associated with topographical distribution (Brown *et al*. 2007). If the three different subspecies found in Kenya are considered, there are clear geographical boundaries, which furthermore suggest reproductive isolation among the populations (Brown *et al*. 2007). Animals migrate between habitats during their lifespan. If a single population stayed in the same habitat for many generations, there would possibly be consequences on the genetic diversity of the population (Lacy 1987). By constantly moving around and occupying various habitats, populations share genetic material, and therefore expand the rate of gene flow within the species (Allendorf and Luikart 2007).

Brown *et al*. (2007) stated that giraffe population distributions in Africa can extend over several hundred square kilometres (km²), with home range sizes between 5km² and 992km² in size. These large home ranges can contribute to low levels of differentiation among the populations due to uninterrupted gene-flow. Deacon (2015), focusing on giraffe home ranges using GPS satellite collars, found that home ranges vary between 177km² and 245km² depending on seasonal conditions.

In research done by Brown *et al*. (2007), skin biopsies were sampled from 266 free-ranging giraffe, from different subspecies, with assignment to specific subspecies based on geographic location and pelage, as described by Dagg and Foster (1982). Genetic structure of the population was assessed by grouping the localities of the various samples together, based on genetic differences. The results revealed

extensive population genetic structure in both mitochondrial and nuclear DNA markers. The classification of the giraffe as a single species can thus have consequences on the conservation of the various subspecies. Due to many external pressures, many subspecies face the risk of dying off if reclassification is not done urgently.

The giraffe occurring in Namibia are classified as *Giraffa camelopardalis angolensis* (Brenneman *et al*. 2009a). In a study done by Brenneman *et al*. (2009a), levels of genetic diversity in populations of the Namibian giraffe were studied using microsatellite loci. For that study, tissue samples were collected from two different giraffe subpopulations found in the northern Namib Desert and Etosha National Park, which are approximately 400km apart. Results showed that both populations had low levels of genetic diversity when compared to other populations found in Africa. The low levels of genetic diversity is explained by Brenneman *et al*. (2009a) as possibly the result of a migration which had occurred between Namibia and Angola, which could include a possible geographical barrier resulting in the isolated population. A closer look at the two subpopulations suggested that there was regular gene flow amongst the northern Namib Desert subpopulations, whereas in contrast there was little genetic evidence that there was regular gene flow amongst the Etosha National Park subpopulations (Brenneman *et al*. 2009a). The translocation of animals from one population to another would allow for an increase in the levels of genetic diversity, however, if human development should restrict the migration of individuals between populations, genetic diversity could be lost as a whole, and inbreeding may occur (Allendorf and Luikart 2007).

1.4 Suitable markers for population genetic studies on giraffe

Various categories of molecular markers can be used to describe genetic diversity and differentiation within and between populations. In a study done on genetic diversity in animals, using mitochondrial DNA (mtDNA) markers, Bazin *et al*. (2006) showed that the use of mtDNA markers does not provide data on the abundance or demographics of a species.

In order to make up for what the mtDNA markers lack, nuclear markers are needed. Nuclear markers not only detect hybridization events originating from movements of both sexes, but also are necessary to describe short-term population genetic processes (Loew 2002). As explained by Loew (2002), microsatellite DNA fingerprinting is ultimately suited for providing data on allele frequencies needed for estimation of gene flow, as well as determination of levels of genetic diversity.

Loew (2002) states that microsatellite DNA reveals genetic differences within individuals and between individuals of the population, providing information on the identity of the individual, parentage determination and genetic diversity; as well as information on intraspecific genetic processes among populations, such as gene flow. It is also explained that mitochondrial DNA (mtDNA) provides information relating to genetic distances among populations and closely related species.

1.4.1 Mitochondrial DNA versus nuclear DNA

Mitochondrial DNA is shown in Figure 1.7 to be circular in structure, whereas nuclear DNA is linear. The mode of inheritance of mtDNA is maternal, in comparison to that of nuclear DNA which is inherited from both parents. Mitochondrial DNA degrades slower than nuclear DNA, and evolves at a much faster rate as well (Arif and Khan 2009). Mitochondrial DNA molecules do not undergo recombination, thereby making these markers valuable for reconstructing phylogenetic trees (Allendorf and Luikart 2007). Microsatellites are randomly distributed throughout the genome and show high levels of polymorphism, resulting from higher mutation rates compared to other nuclear regions (Allendorf and Luikart 2007). Microsatellite alleles show codominant inheritance and miniscule amounts of template DNA are needed for microsatellite genotyping (Di Fiore 2003).

Since microsatellite primers specific to giraffe have not been published or made available, and considering the wide-spread use of mtDNA in conservation genetics, the latter category of markers was considered appropriate for the present study.

1.4.2 Mitochondrial (mtDNA) DNA markers

Mitochondrial DNA is haploid and is inherited maternally by most species (Allendorf and Luikart 2007). This type of DNA is a circular molecule (Figure 1.7) which contains 37 genes and a control region (Loew 2002). According to Loew (2002), the analysis of mtDNA fragments is useful to detect polymorphism which is used to resolve distances among conspecific populations and closely related species. Arif and Khan (2009) stated that mtDNA is best suited to study evolutionary relationships and biodiversity. Protein coding genes of the mitochondrial genome that are mostly used for molecular analysis include cytochrome *b* (Cyt *b*), NADH dehydrogenase subunit 5, and cytochrome oxidase I (COI) (Arif and Khan 2009). In addition, the control region or D-loop is widely used. Here, I will focus on the use of the Cyt *b* and D-loop regions.

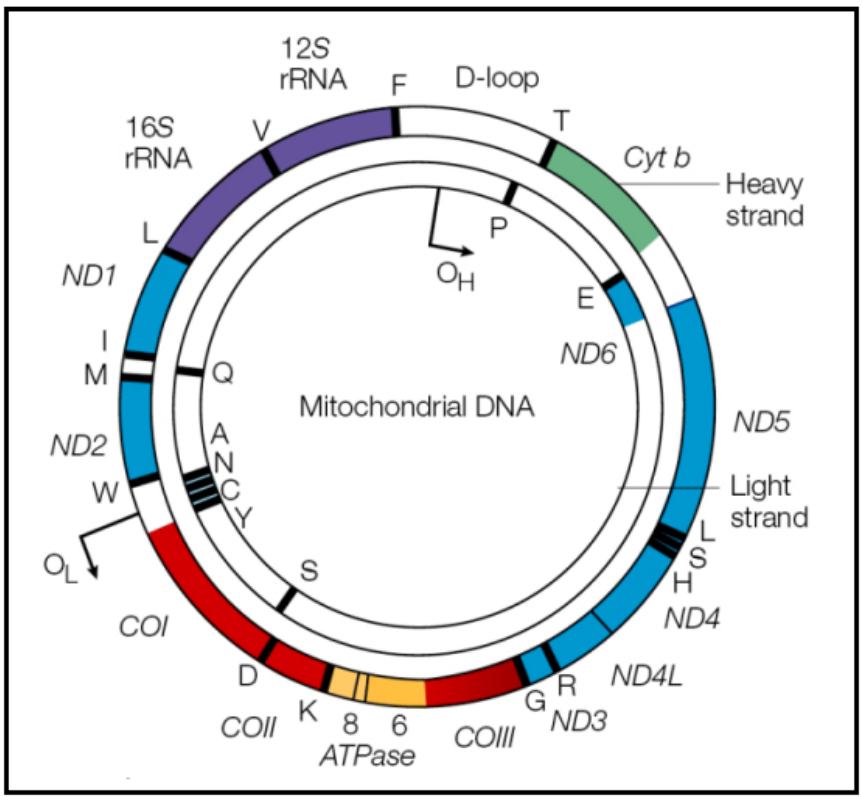

Figure 1.7 A diagrammatic scheme of the Mitochondrial DNA of *Giraffa camelopardalis*. Illustration from Taylor and Turnbull (2005).

1.4.2.1 Studies done using mitochondrial DNA markers

In the study done by Brown *et al*. (2007) on population genetic structure of giraffe, 266 giraffe individuals, as well as one okapi individual were sampled. The aim of the study was to present a phylogeographic and population genetic analysis of the giraffe across most of the species' remaining geographic range. This was done by assessing the genetic variation in mitochondrial sequences, as well as microsatellite loci. From the 266 sequences obtained, the mtDNA data matrix was collapsed into 35 haplotypes. The data from mitochondrial and nuclear DNA markers showed that there was extensive population genetic structure in the individuals sampled. The results of the study indicated that some of the genetically distinct populations represent evolutionary significant units which are highly threatened and do not have the necessary recognition in current management plans.

In a recent study by Bock *et al*. (2014), tissue samples were collected from 161 individuals of various giraffe subspecies located in Botswana, Namibia, and the Democratic Republic of Congo. The aim of the study was to present population genetic

analysis of the mitochondrial cytochrome b gene region, as well as the partial control region, for eight of the nine currently described giraffe subspecies. Mitochondrial cytochrome *b* (Cyt *b*) and partial mtDNA control region (CR) DNA sequences were amplified and analysed, resulting in a dataset based on 1 140bp of the Cyt *b* gene and 422bp of the control region. The results obtained showed that the distribution ranges of the Angolan and South African giraffe need to be redefined, as accurate information on the phylogenetic and genetic variation of these particular subspecies is needed for the development of appropriate management strategies.

1.4.2.2 Cytochrome *b* (Cyt *b*) gene region

This is the most extensively used protein-coding gene of the mitochondrial genome for molecular analysis (Loew 2002). This gene has been used in studies to better understand genetic diversity for improved conservation management of species (Arif and Khan 2009).

1.4.2.3 D-loop region within the Control Region

The mitochondrial control region (CR) is linked with processes such as replication and transcription, and comprises of non-coding DNA in all taxa (Loew 2002). The D-loop segment displays higher levels of variability in comparison to that of protein-coding sequences (Arif and Khan 2009). Loew (2002) states that due to the high variability levels that can be found, this region is the most suitable marker for comparisons of sequences within and between populations and closely related species. The D-loop region has been widely used in studies to measure the molecular diversity and to identify conservation units for better management of species, i.e. to describe population structure and gene flow (Arif and Khan 2009).

1.5 Rationale for the present study

Giraffe populations are often fragmented. In order to conserve a species as a whole, the conservation of individual populations in fragmented population groups should be of high importance. Giraffe have been listed as “Least Concern” on the Red Data list

(Fennessy and Brown 2010), and more recently listed as “Vulnerable” by Muller *et al*. (2016), however two subspecies have been listed as “Endangered”. In general, giraffe numbers seem to be declining, and this is due to multiple factors. With little being known about the effect of isolation and fragmentation in this species, the aim of the current project was to study levels of genetic diversity within and between individual giraffe populations, as well as to determine the genetic structure of *Giraffa camelopardalis* in Central South Africa; specifically the position of the giraffe in this region relative to the wider southern African population.

In order to determine levels of genetic diversity within and between fragmented giraffe populations, two regions of the mtDNA genome were sequenced in giraffe from several populations. In this first study focussed on the genetic diversity of giraffe populations within the Free State Province, South Africa, I looked at the management histories of the different giraffe populations, as well as the current genetic structure. I aimed to estimate the effects of isolation and fragmentation on the populations, as well as the genetic structure within the Free State Province and identify possible sources of introductions into the province.

There are two specific aims for this study. The first aim of this study is to determine the levels of genetic diversity within individual giraffe population, in order to determine the effect of isolation and fragmentation. If levels of genetic diversity are low, management history should be considered. Secondly, it would be aimed to determine the genetic structure of *Giraffa camelopardalis* in the Free State. With little to no knowledge being available about the giraffe in the Free State, any information regarding this situation would prove to be valuable.

Based on the preceding literature review, the following two hypotheses were identified for the present study:

Hypothesis 1: Genetic diversity in giraffe populations is correlated to the degree of isolation.

Hypothesis 2: The extralimital giraffe population of Central South Africa was founded from one subspecies.

1.6 Dissertation outline

Following the introduction to the evolution, biology and management of giraffe in Chapter 1, methods and materials that were used for this study (detailing the fieldwork and laboratory work done) are described in Chapter 2. Chapter 3 will present the results obtained from the laboratory analysis of the various giraffe populations found in the Free State Province, with particular focus on the cytochrome *b* (Cyt *b*) gene and the D-loop segment within the control region of the mitochondrial genome. The results are discussed in Chapter 4, with reference to the effect of management history on current patterns of diversity and differentiation, and management implications. Chapter 4 includes the conclusion of the study, with additional recommendations for future studies.

Chapter 2.

Materials and Methods

2.1 Samples used

Giraffe faecal (dung) samples were collected from 20 localities within the Free State Province of South Africa (Figure 2.1); with a total number of 129 faecal samples collected. A further three tissue samples stored in formalin from giraffe in the Free State, and 17 blood samples from giraffe in the Northern Cape (Figure 2.2) – stored at the UFS – were also included in the study. These samples were previously collected by Dr. F. Deacon during a study on the collaring of giraffe, as part of an interdisciplinary study in order to understand the biological and ecological factors that regulate giraffe. The total number of samples available for analysis was thus 132 from the Free State, with 17 samples from the Northern Cape.

As reference material, the full range of giraffe sequences available from GenBank (Benson *et al*. 2005) at the time of writing was also included, in order to provide a broader perspective of the genetic diversity of giraffe, as well as for comparative purposes.

The methods employed during this study were approved by the Interfaculty Animal Ethics Committee of the University of the Free State (Student Project number: UFS-AED2015/0050). Collection of faecal samples were sanctioned under a permit issued by the Free State Department of Economic, Small Business Development, Tourism and Environmental Affairs (Permit number: 01/30305). These samples were collected under the approval from the Interfaculty Animal Ethics Committee of the University of the Free State (Student Project number: UFS-AED2015/0066). Section 20 veterinary approval for the collection, transport and storage of samples was obtained from the National Department of Agriculture, Forestry and Fisheries (DAFF), with approval number 12/11/1/4. All tissue and blood samples used were obtained from other sources, thereby not personally obtained. These samples were collected under appropriate permits issued by the Northern Cape Department of Environment and Nature Conservation (Permit number: FAUNA 0729/2017 and FAUNA 0730/2017).

2.1.1 Populations sampled for the present study

Sample sizes and the various localities where the populations were sampled for this study are listed in Table 2.1 and are indicated in Figures 2.1 and 2.2. Sites and samples from individuals were coded to ensure anonymity of private and public owners of giraffe populations.

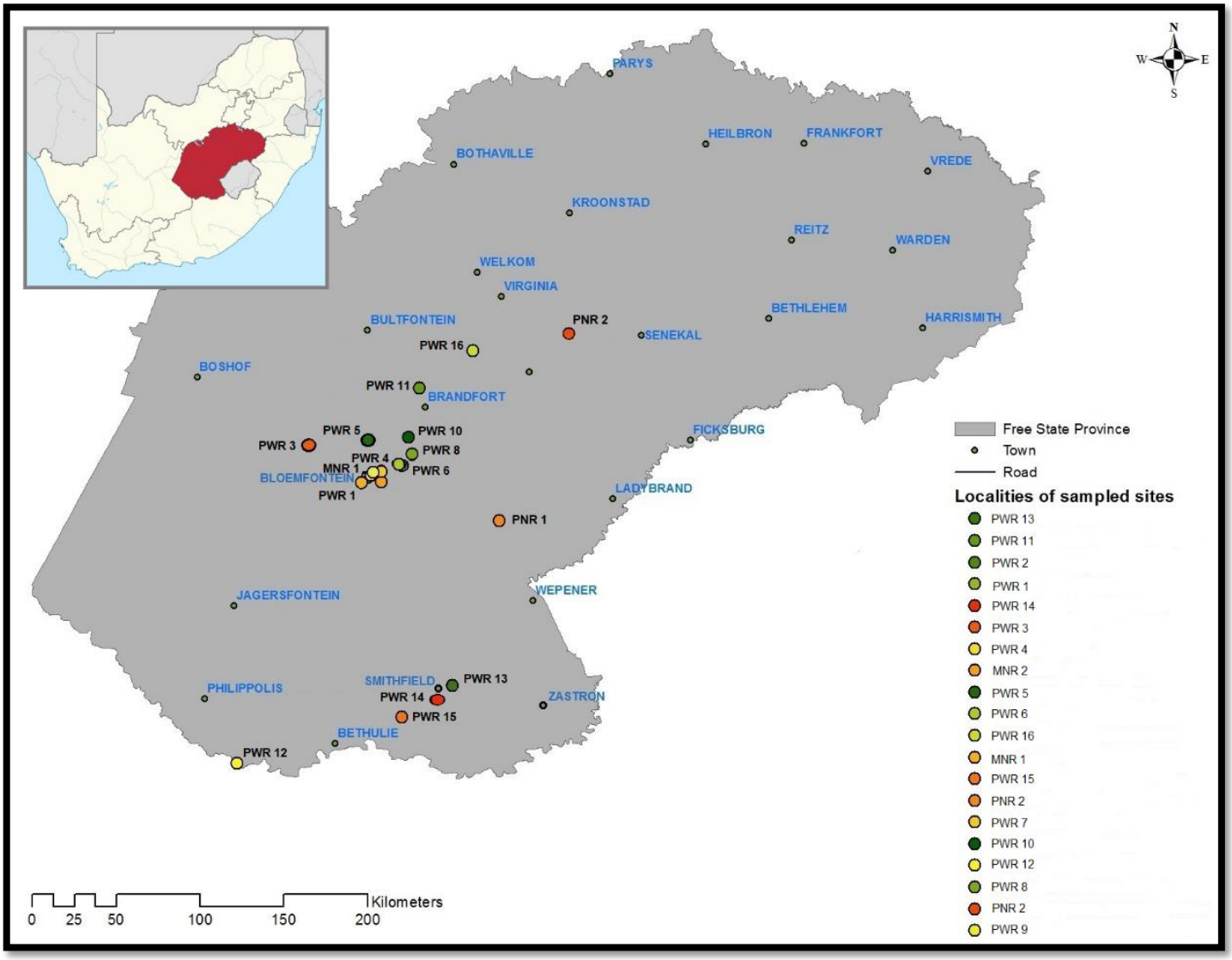

Figure 2.1 A map showing all the Free State localities sampled during this study.

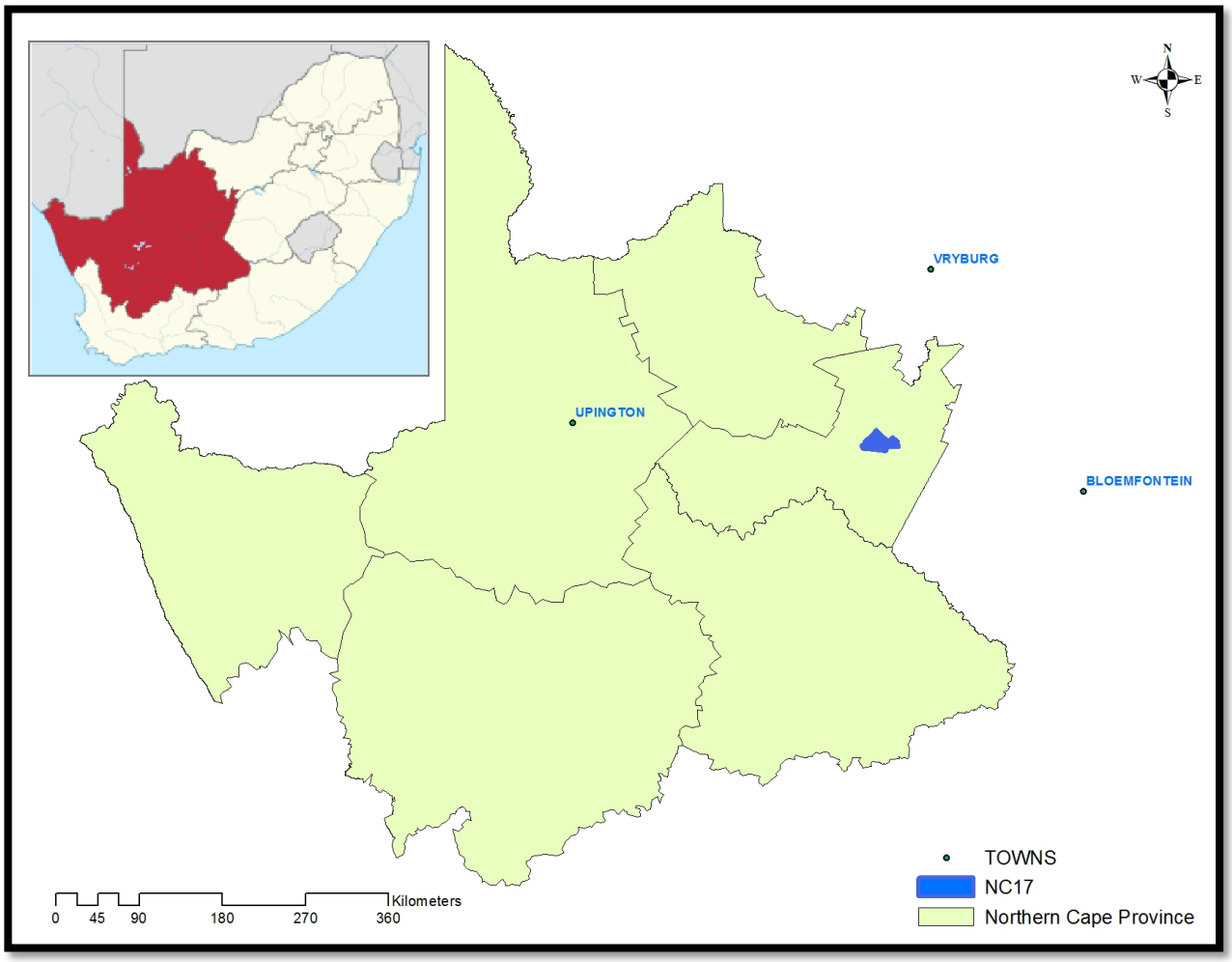

Figure 2.2 A map showing the Northern Cape locality sampled during the current study.

Table 2.1 A list of the sample numbers and origin of all faecal samples used for this study.

Sample Number	Locality	Area	Census Size (Sample Size)	Sample History
MNR 1_01 – MNR 1_02	Municipal Nature Reserve 1 (MNR 1)	Bloemfontein	2 (2)	This reserve is located just outside the city of Bloemfontein. Two giraffe (one bull and one cow) were sampled. These two giraffe were originally translocated from the Johannesburg Zoo.
MNR 2_01 – MNR 2_03	Municipal Nature Reserve 2 (MNR 2)	Bloemfontein	2 (2)	This reserve is located in the Bloemfontein area. Two giraffe (one bull and one cow) were sampled. Data on the management history of these giraffe is limited - the cow is the mother of the bull. A tissue sample was also obtained from a deceased bull previously, and this is the father of the living bull.
PNR 1_01 – PNR 1_02	Provincial Nature Reserve 1 (PNR 1)	Thaba Nchu	2 (0)	This reserve is located near the town of Thaba Nchu, approximately 80km from Bloemfontein. Two giraffe (one bull and one cow) were sampled. The giraffe from this reserve were translocated from the population in Provincial Nature Reserve 2 .
PNR 2_01 – PNR 2_33	Provincial Nature Reserve 2 (PNR 2)	Senekal	21 (17)	This reserve is located near the town of Senekal, approximately 158km from Bloemfontein. A total of 21 giraffe were sampled, and one sample from a deceased bull was previously taken. The giraffe from this Nature Reserve originated from various localities over the years, namely from the Limpopo Province SPCA, Hans Merensky Reserve in Limpopo, as well as the Weenen area in Kwa-Zulu Natal.
PWR 1_01	Private Wildlife Reserve 1 (PWR 1)	Bloemfontein	1 (1)	A single giraffe cow was sampled at Private Wildlife Reserve 1. The reserve is situated in the Bloemfontein area. The parents of the cow were originally bought approximately 200km north of Bloemfontein, and translocated to the current locality. The current cow was a direct offspring from these adults. Subsequently, after sample collection, the cow has been translocated to Private Wildlife Reserve 7.
PWR 2_01 – PWR 2_06	Private Wildlife Reserve 2 (PWR 2)	Bloemfontein	7 (5)	This reserve is located in the Bloemfontein area. A total of five giraffe were sampled from this locality. The management history of these giraffe are unknown.
PWR 3_01 – PWR 3_10	Private Wildlife Reserve 3 (PWR 3)	Bloemfontein	12 (10)	Private Wildlife Reserve 3 is located approximately 40km from Bloemfontein. A total of 10 giraffe were sampled. The management history of these giraffe is unknown.
PWR 4_01 – PWR 4_02	Private Wildlife Reserve 4 (PWR 4)	Bloemfontein	2 (2)	Private Wildlife Reserve 4 is located in the Bloemfontein area. Two giraffe (one bull and one cow) were sampled. The management history of these giraffe is unknown.
PWR 5_01 – PWR 5_02	Private Wildlife Reserve 5 (PWR 5)	Bloemfontein	2 (2)	This reserve is located approximately 30km from Bloemfontein. Two giraffe (one bull and one cow) were sampled. The management history of these giraffe is unknown.
PWR 6_01 – PWR 6_08	Private Wildlife Reserve 6 (PWR 6)	Bloemfontein	15 (7)	Private Wildlife Reserve 6 is located in the Bloemfontein area. A total of seven giraffe were sampled. The management history of some of the giraffe is known: four giraffe were translocated from a location in Bloemfontein, and another two giraffe

				were translocated from the Private Wildlife Reserve 3 population.
PWR 7_01	Private Wildlife Reserve 7 (PWR 7)	Bloemfontein	1 (1)	A single giraffe cow was sampled at Private Wildlife Reserve 7. The reserve is situated in the Bloemfontein area. The management history of the locality is unknown, except for the information that the bull was purchased in September 2016 from a location north of Bloemfontein.
PWR 8_01 – PWR 8_03	Private Wildlife Reserve 8 (PWR 8)	Bloemfontein	5 (3)	Private Wildlife Reserve 8 is approximately 35km from Bloemfontein. A total of three giraffe were sampled. The management history of these giraffe is unknown.
PWR 9_01 – PWR 9_07	Private Wildlife Reserve 9 (PWR 9)	Bloemfontein	10 (7)	This reserve is located in the Bloemfontein area. A total of seven giraffe were sampled. The original giraffe were bought in 2002 and have gone on to produce offspring. The geographic origin of the founder population is however not known.
PWR 10_01 – PWR 10_03	Private Wildlife Reserve 10 (PWR 10)	Brandfort	3 (3)	Private Wildlife Reserve 11 is located near Brandfort, and is situated approximately 30km from Bloemfontein. A total of three giraffe were sampled. The bull and cow from this reserve were translocated from a location near Brandfort, and have gone on to produce offspring, of which the calf sampled is one.
PWR 11_01 – PWR 11_37	Private Wildlife Reserve 11 (PWR 11)	Brandfort	37 (35)	This reserve is located near the town of Brandfort, and is situated approximately 75km from Bloemfontein. A total of 30 giraffe were sampled, and one sample from a deceased bull was previously taken. The management history of the giraffe is unknown; however, it is known that giraffe were bought over a span of several years, from different localities.
PWR 12_01 – PWR 12_02	Private Wildlife Reserve 12 (PWR 12)	Gariep Dam	2 (2)	Private Wildlife Reserve 12 is located near the town of Gariep dam, approximately 185km from Bloemfontein. Two giraffe (one bull and one cow) were sampled. The management history of these giraffe is unknown.
PWR 13_01	Private Wildlife Reserve 13 (PWR 13)	Smithfield	1 (1)	This reserve is located near the town of Smithfield, approximately 145km from Bloemfontein. A single giraffe bull was sampled. The giraffe from this reserve and Private Wildlife Reserve 14 originate from the same population, located near Kimberley in the Northern Cape Province.
PWR 14_01	Private Wildlife Reserve 14 (PWR 14)	Smithfield	1 (1)	Private Wildlife Reserve 14 is located near the town of Smithfield, approximately 160km from Bloemfontein. A single giraffe cow was sampled. The origin of this animal is as described above, i.e. it originated from the Kimberley area.
PWR 15_01	Private Wildlife Reserve 15 (PWR 15)	Smithfield	1 (1)	This reserve is located near the town of Smithfield, approximately 180km from Bloemfontein. A single cow was sampled. The management history of the giraffe was not obtainable; however, it is known that two giraffe were originally bought to found the population.
PWR 16_01 – PWR 16_02	Private Wildlife Reserve 16 (PWR 16)	Theunissen	3 (2)	Private Wildlife Reserve 16 is located near the town of Theunissen. Two giraffe (one bull and one cow) were sampled. The management history of these giraffe is known, with the bull translocated from the

				Sandveld Nature Reserve population and the cow from the Philippolis area.
PWR 17_01 – PWR 17_17	Private Wildlife Reserve 17 (PWR 17)	Kimberley	20 (17)	This reserve is located in the Northern Cape, near Kimberley. A total of 17 giraffe were sampled, where six were bulls and 11 were cows. The management history of the reserve is known: there were originally 62 founding giraffe. In 1964, seven giraffe were bought from Letaba, however, they died due to the fact that they were hand-reared when bought, and could not adapt to browsing on their own. In 1971, eight giraffe were bought from Grootfontein in Namibia, where another six giraffe were bought in 1973 from Transvaal Game Breeders.

2.1.2 Sampling collection techniques

Non-invasive sampling was done in order to minimize stress on the individuals, as well as for cost purposes and due to ethical considerations. Samples were located by observation and spotting. The procedure followed at each locality was as follows: once a giraffe was found at the various locations, observation of the giraffe started. When defecation had occurred, the exact spot was marked, and retrieval of the faecal samples happened within an hour. Each giraffe was also carefully identified using patterns on its body, to ensure that individuals were not mistakenly sampled twice. Faecal samples were stored in 15ml blue-top tubes, filled with 96% ethanol, for preservation purposes (Figure 2.3). The Sample Number, Sex, Date of Collection, Locality, as well as Sample Type was labelled on each blue-top tube. The collected samples were then placed in a freezer for further preservation at -20˚C as soon as possible, until DNA extraction could take place.

Figure 2.3 Collection of giraffe faecal samples by M.E. van Niekerk.

2.1.3 GenBank samples

Relevant DNA sequences, i.e. for the same gene regions used during the present study, were downloaded from GenBank. All of the reference sequences were results from various studies on the giraffe subspecies composition. An outgroup consisting of Okapi sequences of the specific genes used for this study, was also downloaded for comparative purposes. The okapi is closely related to the giraffe, and these species share a common ancestor. A total of 151 reference sequences were downloaded for the Cyt *b* gene region, and 177 reference sequences were downloaded for the D-loop region.

2.2 DNA extraction

Before each step of the process (DNA Extraction, Gel Electrophoresis, Polymerase Chain Reaction, etc.) was performed, the work benches were cleaned with bleach, all items used rinsed off with water, all necessary calculations for conversions were done, and gloves and a laboratory coat were worn. All tubes were correctly marked with a permanent marker, with all necessary information pertaining to the sample and its origin.

2.2.1 DNA isolation

DNA was extracted using three different extraction kits, suited to the source material.

2.2.1.1 DNA isolation from faecal samples

DNA extraction from faecal samples were done using a Zymo Research ZR Fecal DNA MiniPrep™ kit. The standard protocol of the specific kit was used, with no adjustments made to any of the steps.

2.2.1.2 DNA isolation using tissue samples

DNA extraction from tissue samples were done using a Zymo Research Quick-DNA™ FFPE MiniPrep kit. No adjustments were made to any of the steps while following the standard protocol of the specific kit.

2.2.1.3 DNA isolation using blood samples

DNA extraction from blood samples were done using a Roche High Pure PCR Template Preparation kit. For the process, the standard protocol of the specific kit was used, with no adjustments made to any of the steps.

2.2.2 Verification of DNA quality and quantity

2.2.2.1 Spectrophotometry

A NanoDrop® ND-1000 Spectrophotometer was used to determine the quantity and quality of extracted DNA. Distilled water was used to clean the loading surfaces of the NanoDrop®. Once the spectrophotometer was calibrated (using water and elution buffer samples), 1 µl of eluted nucleic acid was dropped onto the pedestal, and the quantity and quality of the nucleic acid was recorded (in ng/µl and using the 260/280 absorption ratio respectively).

2.2.2.2 Gel electrophoresis

Once the NanoDrop-based analyses were done, gel electrophoresis was performed as an additional check of DNA quality. A total of 80g of Agarose powder was added to 800ml of Tris-acetate-EDTA buffer (TAE) in a glass bottle which had a screw-on lid. The mixture was then placed into a microwave until the solution became clear. Once clear, the lid was tightly screwed on and the bottle was cooled under running water until the temperature was cool enough for the bottle to be able to work with (the “baby bottle method”). The solution was then poured into the gel container, which already had the combs inserted into it. The solution in the gel container was left for approximately 20 minutes in order to set. Once set, the combs were removed, and the gel was inserted into the electrophoresis system with the wells closest to the positive electrode. Enough TAE Buffer was poured into the system in order to cover the gel. A small piece of Para film was then cut. The Para film was used to mix the DNA and the loading Buffer. A 2 µl droplet of Loading Buffer (Gel Red) was dropped onto the Para film for each DNA sample, before being mixed with 4 µl of the eluted nucleic acid. The mixture was then loaded into a well. The gel was then run on the Bio Rad PowerPac™ at 100 V for 25 minutes. After electrophoresis, the gel was removed from the system, and placed onto the UV-box for visualization. Photos of the gel were taken using the Syngene G:Box system, to provide a permanent record.

2.2.3 Sequencing of the Cyt *b* and D-loop mtDNA regions:

The procedure to sequence both the Cyt *b* and D-loop mtDNA regions consisted of Pre-sequence PCR, cleaning of PCR products, PCR for sequencing, and clean-up for sequencing.

The Cyt *b* primers used were from a study by Bock *et al.* (2014), with the sequences of primers used for the D-loop region sourced from a study by Seymour (2001).

The primer sequences for the various gene regions were as follows:

- Cyt *b* forward primer 5'-GTGGAAGGCGAAGAATCG -3' and Cyt *b* reverse primer 5'-TGAAAAACCATCGTTGTCGT-3' (Bock *et al.* 2014).
- D-loop forward primer 5'-CCCAAAGCTGAAGTTCTATT-3' and D-loop reverse primer 5'-CAATAACTGTATGTACTATG-3' (Seymour 2001).

2.2.3.1 Pre-sequence Polymerase Chain Reaction (PCR)

For pre-sequence PCR for the Cyt *b* gene region, a 1.5 ml tube was used to prepare the stock solution (master mix). The stock solution was based on a Kapa2G™ Robust HotStart ReadyMix PCR Kit, with the forward and reverse primers, and nuclease free water added. For each 12.5 µl volume reaction mixture, 6.25 µl ReadyMix, 0.625 µl of each primer (10 pmol), and 2 µl distilled water (dH_2O). After the stock solution was prepared, 9.5 µl was pipetted into each of a number of 0.2 ml PCR tubes. A volume of 3 µl of 50-200 ng/µl of template DNA was then added to each PCR tube, with the tube then centrifuged. The amplification conditions were as follows: 3 minutes at 95ºC for initial denaturation; followed by 35 cycles, each of 15 seconds at 95ºC, 15 seconds at 62ºC, and 15 seconds at 72ºC; followed by final elongation of 10 minutes at 72ºC, and then 12ºC indefinite hold.

For pre-sequence PCR for the D-loop region, the stock solution was based on the Ampliqon TEMPase Hot Start 2x Master Mix A Kit, with the forward and reverse primers and nuclease free water added. For each 12.5 µl volume reaction mixture, 6.25 µl ReadyMix, 0.25 µl of each primer (10 pmol), and 3.5 µl distilled water (dH_2O). Once the stock solution was prepared, 10 µl was pipetted into each of several 0.2 ml PCR tubes. A volume of 2.5 µl of 1-200 ng/µl of template DNA was then added to each PCR tube, with the tube then centrifuged. The amplification reaction followed the following PCR protocol: 15 minutes at 95ºC for initial denaturation; followed by 35 cycles, each of: 25 seconds at 95ºC, 30 seconds at 50ºC, and 60 seconds at 72ºC; followed by final elongation of 5 minutes at 72ºC, and then 12ºC indefinite hold.

2.2.3.2 Amplification and sequencing of the Cyt *b* and D-loop regions

Before PCR Purification, the pre-sequence PCR product was examined by gel electrophoresis in order to determine whether amplification had occurred, and if there was product be purified.

2.2.3.3 PCR purification

Purification of satisfactory quality products was performed with the Biospin PCR Purification Kit (Bio-Rad). This specific kit was used to remove primers which were present in the PCR products and could thus only be used if the DNA fragments were larger than 50bp. A volume of 8.5 µl of the PCR product was transferred to a 1.5 ml microcentrifuge tube. Two volumes (17.0 µl) of Binding Buffer was then added to 1 volume (8.5 µl) of the PCR product and the tube was vortexed. The mixture was then pipetted from the microcentrifuge tube into the provided spin column and centrifuged for 1 minute at 9 000 rpm. The flow-through was then discarded and a new collection tubes was attached to the spin column. Next 650 µl wash buffer was added to the equivalent spin columns, and yet again centrifuged for 1 minute at 12 000 rpm, before discarding the flow-through again and replacing the collection tubes. The previous step regarding the wash buffer was then repeated once. The spin column was attached to another collection tube and then centrifuged for another minute at 12 000 rpm, before transferring the spin column to a sterile 1.5 ml microcentrifuge tube. A volume of 35 µl elution buffer was then added to the spin column and this was left to stand for a minute at room temperature. It was then centrifuged for a further minute at 12 000 rpm. The microcentrifuge tube then contained the purified template DNA that was stored at -20°C.

2.2.3.4 Sequencing reactions

A method based on the ABI PRISM® BigDye® Terminator v3.1 Cycle Sequencing Kit was used. The procedure for PCR for sequencing was based on two stock solutions being made - one for the forward primer and one for the reverse primer. The primers used for the various mtDNA regions were as before, but with additional dilution to 2

mM. Both stock solutions contained, for each reaction to be done: 0.5 µl BigDye® Terminator mix, 2.5 µl BigDye® Terminator v3.1 sequencing buffer, 4 µl dH_2O and 1 µl of the applicable primer (at 2mM). All the chemicals used in the stock solution were stored in a box filled with ice. Once the stock solutions were prepared, 8 µl was pipetted into each 0.2 ml PCR tube. A volume of 2 µl of the PCR product was then added to each PCR tube, before being vortexed and centrifuged. The reactions then followed the following PCR program: 94 ºC for 2 minutes for initial denaturation, a cycle of 34 times (15 seconds at 94ºC, 10 seconds at 53ºC, and 3 minutes at 60ºC), followed by an indefinite hold at 12ºC.

2.2.3.5 Post sequencing clean-up

This procedure was performed using the ZR DNA Sequencing Clean-up™ Kit. For clean-up for sequencing, 240 µl of sequencing binding buffer was added to a 10 µl sequencing reaction. The mixture was then transferred to a Zymo-Spin™ IB Column in a collection tube and centrifuged at 12 000 rpm for 30 seconds. After centrifugation, 300 µl sequencing wash buffer was added to each column, before centrifuging at 12 000 rpm for another 30 seconds. The column was then centrifuged for an additional minute at 12 000 rpm. The column was then transferred to a 1.5 ml microcentrifuge tube, with 12 µl HiDi then added directly to the column matrix before centrifugation for 15 seconds at 12 000 rpm in order to elute the DNA. The ultra-pure DNA was then ready to be loaded into the ABI 3130 sequencer.

The Zymo-Spin™ IB columns and collection tubes could be used up to ten times and were therefore cleaned using the following procedure: add 500 µl of 0.1% Hydrochloric acid (HCl) and leave to stand in room temperature for 30 minutes. After standing for 30 minutes, the columns and collection tubes were centrifuged at 13 000 rpm for a minute. Then were then left to dry overnight, before being available for use again.

2.2.4 Genetic analysers used

Prepared samples were loaded onto either an ABI 3130 or ABI 3500 Genetic Analyser for Sanger-sequencing.

2.3 Statistical analysis

2.3.1 Sequence assembly and alignment

Geneious Pro v4.7.4 software (Drummond *et al*. 2009) was used to view, assemble and align all raw genetic sequence files (.ab1 format) received from the Genetic Analyser. The Geneious software enables the researcher to perform tasks such as sequence assembly and mapping, alignment, phylogenetic analysis and primer design. Raw genetic sequences were first scanned visually as an initial quality control, before proceeding with assembly. The parameters used for assembly of the sequences are listed in Table 2.2.

Once the sequences were assembled, a sequence blast was performed using the National Centre for Biotechnology Information (NCBI) Basic Local Alignment Search Tool (BLAST) (Altschul *et.al*. 1990), to verify sequence identity with the same species and gene region from other studies. Assembled sequences were then aligned using ClustalW, as implemented in Mega v7.0.26 software (Kumar *et al*. 2015). Once aligned, the sequences were trimmed to a length of 405bp for the Cyt *b* gene, and 267bp for the D-loop region, to ensure that all sequences were of same length during analysis. The Mega software allows the researcher to build and edit sequence alignments, test various models of substitution which could be suitable to the study, compute distance and diversity, construct phylogenetic trees, and determine rates of sequence divergence. The parameters used for the sequence alignments during the study are listed below in Table 2.3. For both mtDNA regions used, additional reference sequences were added from NCBI nucleotide bank (GenBank) (Benson *et al*. 2005) for the various giraffe subspecies, as well as *Okapia johnstoni* sequences as an outgroup.

Table 2.2 Assembly parameters used for DNA assembly and alignment using Geneious Pro v4.7.4.

Assembly Parameters:	
Assembly Options:	
Minimum Overlap	25
Overlap Identity %	80
Alignment Options:	
Gap Open / Extend Penalty	18
Mismatch Score	-9
Match Score	5

Table 2.3 Alignment parameters used for DNA alignment using ClustalW in Mega v7.0.26.

Alignment Parameters:	
Pairwise Alignment:	
Gap Opening Penalty	15
Gap Extension Penalty	6.66
Multiple Alignment:	
Gap Opening Penalty	15
Gap Extension Penalty	6.66
DNA Weight Matrix	IUB
Transition Weight	0.5
Use Negative Matrix	OFF
Delay Divergent Cut off (%)	30

2.3.2 Genetic diversity

2.3.2.1 Genetic diversity within populations

Diversity within each of the 21 populations studied was quantified using the number of haplotypes within the population (h), the haplotype diversity (H_d), and the nucleotide diversity (π) (Nei 1987). DnaSP v5 software (Librador and Rozas 2009) was used to calculate these coefficients of diversity within populations.

Many of the populations studied during the current investigation were small and were expected to contain low levels of genetic diversity. It is also expected the phenomenon of keeping small giraffe populations is unlikely to change. Therefore, it was considered beneficial to investigate the level of genetic diversity in pooled "metapopulations". Data from the populations sampled were thus pooled, using two

approaches. First, samples from all the small privately owned reserves, but excluding the largest of the private reserves, as well as all public reserves, were pooled (SPFSp). As a second scenario, all the populations sampled in the Free State Province were pooled, to gauge the overall level of diversity in a potential Free State provincial metapopulation (AFSp).

2.3.3 Genetic differentiation between populations

For the analysis of genetic differentiation between specific groups, populations and subspecies were grouped as follows: Free State, Northern Cape, and the available reference material for each subspecies.

Differentiation among populations based on F_{ST} was calculated using Arlequin v3.5.2.2 (Excoffier 2015). To determine whether the obtained F_{ST} values indicated significant differentiation between population pairs, a significance level of 0.05 was used. Genetic divergence between populations were quantified using the average number of nucleotide differences between populations, the average number of nucleotide substitutions per site between populations (D_{xy}), and the number of net nucleotide substitutions per site between populations (D_a). The diversity measures were formulated by Nei (1987), and calculated using DnaSP v5 software.

2.3.4 Molecular phylogenetic analysis

2.3.4.1 Phylogenetic analysis

Mega v7.0.26 software was utilised to identify the best model of substitution to use when constructing the phylogenetic trees. Phylogenetic trees were then constructed using a Maximum Likelihood (ML) approach with the specific model. The phylogenetic analysis was done using a bootstrap method, with 1 000 bootstrap replications. The phylogenetic tree for the Cyt *b* gene was best described by the Kimura 2-parameter model with Gamma Distributed rates among sites (K2+G), whereas substitution at the D-loop region was best described by the Tamura-Nei model with Gamma Distributed rates among sites (TN93+G).

For a Bayesian approach to the study of relationships among populations, BEAUti v1.7 and BEAST software (Drummond *et al*. 2012) were used. The BEAUti software was first to convert a Nexus file (created using Mega v7.0.26) into a XLM document, which is used as input for BEAST software. The length of the chain was set as 1 000 000 steps, with log parameters calculated every 100 steps. The output from BEAST v1.7 was then used as input in TreeAnnotator v1.7 (Drummond and Rambaut 2007) to finally produce a file compatible with FigTree v1.4.3 (Rambaut 2016). The final phylogenetic tree could then be visualised in FigTree v1.4.3, where modification could take place, i.e. zoom, expand and the addition of bootstrap values.

2.3.4.2 Haplotype Network

To complement the phylogenetic analysis, haplotype networks were constructed. In a haplotype network, each node can be connected to multiple nodes, not only to one as in a phylogenetic tree. A haplotype network shows the mutational history amongst the nodes; however, it does not illustrate the ancestry. A phylogenetic tree, in contrast, portrays the ancestral history (Baum 2008).

Network v5.0.0.3 software (Fluxus Engineering 2017) was used to construct the phylogenetic networks using the haplotype data obtained from the study, and using a median-joining approach. The number of mutational steps which had occurred between the various linked haplotypes were indicated in the networks generated. The total number of individuals with each haplotype were also represented, using various colours to indicate the different subspecies, as well as the outgroup and the samples of the current study.

Chapter 3.

Results

3.1 DNA isolation

The majority of DNA used in this study was extracted from faecal samples (89), followed by blood samples (12), and with a small number of tissue samples also used (3). The DNA quantity values were lower in faecal samples (Table 3.1) as compared to the values for the extraction from tissue samples (Table 3.3), but with blood samples yielding the lowest recovery of DNA (Table 3.2). The DNA yield from faecal samples ranged from 7.80 ng/µl to 51.77 ng/µl, with an average of 20.23 ng/µl. By comparison, blood samples yielded only 0.83 ng/µl to 22.67 ng/µl, with an average of 3.40 ng/µl. The quantity of DNA from tissue samples ranged from 77.77 ng/µl to 124.93 ng/µl, with an average of 108.59 ng/µl.

The DNA quality values were lower in blood samples (Table 3.2) as compared to the values for the extraction from tissue samples (Table 3.3), but with faecal samples yielding the lowest quality of DNA (Table 3.1). The DNA quality (260/280 ratio) from faecal samples ranged from 1.16 to 2.25, with an average of 1.37. By comparison, blood sample quality was only 1.32 to 2.97, with an average of 1.91. The quality of DNA from tissue samples ranged from 1.78 to 2.00, with an average of 1.92.

Table 3.1 DNA Quantities (ng/µl) and Qualities (260/280 ratio) extracted from faecal samples.

Sample	Quantity/Quality	Sample	Quantity/Quality	Sample	Quantity/Quality
MNR 1_01	17.80/1.19	**PWR 5_01**	29.07/1.38	**PWR 11_14**	22.73/1.38
MNR 2_02	19.70/1.32	**PWR 5_02**	16.20/1.25	**PWR 11_15**	14.30/1.30
MNR 2_03	15.07/1.25	**PWR 6_01**	24.33/1.37	**PWR 11_18**	7.80/2.25
PNR 2_03	19.87/1.34	**PWR 6_02**	18.03/1.48	**PWR 11_19**	18.50/1.27
PNR 2_07	15.50/1.23	**PWR 6_03**	22.20/1.40	**PWR 11_20**	18.00/1.49
PNR 2_17	23.80/1.31	**PWR 6_04**	21.37/1.46	**PWR 11_21**	18.00/1.40
PNR 2_20	8.43/1.29	**PWR 6_05**	20.03/1.57	**PWR 11_22**	15.30/1.38
PNR 2_21	20.00/1.30	**PWR 6_07**	21.63/1.36	**PWR 11_23**	8.40/1.70
PNR 2_22	14.13/1.37	**PWR 6_08**	18.67/1.50	**PWR 11_24**	17.20/1.33
PNR 2_23	10.47/1.29	**PWR 7_01**	22.43/1.38	**PWR 11_25**	20.57/1.50
PNR 2_25	17.97/1.23	**PWR 8_01**	25.80/1.28	**PWR 11_26**	16.27/1.33
PNR 2_26	19.40/1.28	**PWR 8_02**	22.60/1.39	**PWR 11_27**	20.50/1.32
PNR 2_27	24.07/1.29	**PWR 8_03**	15.30/1.24	**PWR 11_28**	18.77/1.21

PNR 2_28	18.50/1.22	**PWR 9_01**	34.23/1.50	**PWR 11_29**	28.30/1.51
PNR 2_29	20.33/1.18	**PWR 9_02**	23.97/1.32	**PWR 11_30**	22.53/1.41
PNR 2_30	14.27/1.23	**PWR 9_03**	22.17/1.29	**PWR 11_31**	15.53/1.43
PNR 2_31	21.97/1.26	**PWR 9_04**	17.77/1.37	**PWR 11_32**	20.67/1.28
PNR 2_32	21.23/1.42	**PWR 10_02**	22.60/1.28	**PWR 11_33**	32.93/1.52
PNR 2_33	14.23/1.19	**PWR 11_02**	12.47/1.27	**PWR 11_34**	26.17/1.54
PWR 1_01	20.07/1.37	**PWR 11_03**	18.23/1.29	**PWR 11_35**	25.17/1.47
PWR 2_02	21.70/1.50	**PWR 11_04**	17.90/1.27	**PWR 11_36**	30.10/1.73
PWR 2_03	25.57/1.40	**PWR 11_05**	19.80/1.30	**PWR 11_37**	13.57/1.50
PWR 2_04	23.03/1.27	**PWR 11_06**	14.37/1.41	**PWR 12_01**	29.73/1.16
PWR 2_05	21.87/1.27	**PWR 11_07**	17.00/1.33	**PWR 12_02**	19.73/1.30
PWR 2_06	19.17/1.38	**PWR 11_08**	17.07/1.24	**PWR 13_01**	21.73/1.25
PWR 3_01	12.13/1.31	**PWR 11_09**	51.77/1.54	**PWR 14_01**	20.90/1.38
PWR 3_02	18.67/1.39	**PWR 11_10**	11.43/1.32	**PWR 15_01**	28.30/1.32
PWR 3_03	15.07/1.36	**PWR 11_11**	17.07/1.28	**PWR 16_01**	25.57/1.52
PWR 4_01	17.47/1.28	**PWR 11_12**	20.57/1.55	**PWR 16_02**	41.53/1.51
PWR 4_02	19.33/1.26	**PWR 11_13**	14.33/1.37		

Table 3.2 DNA Quantities (ng/µl) and Qualities (260/280 ratio) extracted from blood samples.

Sample	Quantity/Quality
PWR 17_03	2.17/1.94
PWR 17_05	0.83/2.09
PWR 17_06	1.47/1.47
PWR 17_07	1.63/1.74
PWR 17_09	1.80/1.76
PWR 17_10	1.50/1.67
PWR 17_11	1.53/1.32
PWR 17_12	1.80/2.47
PWR 17_14	1.93/2.97
PWR 17_15	1.60/1.93
PWR 17_16	1.90/1.76
PWR 17_17	22.67/1.76

Table 3.3 DNA Quantities (ng/µl) and Qualities (260/280 ratio) extracted from tissue samples.

Sample	Quantity/Quality
MNR 2_01	124.93/1.78
PWR 11_01	77.77/2.00
PNR 2_01	123.07/1.98

3.2 Gene sequences of the Cytochrome *b* and D-loop mtDNA regions

A sequence of 332-578bp was amplified from each giraffe DNA sample for the Cyt *b* region, using the primer pair Giraffa-Cytb Fw and Giraffe-Cytb Rev. The forward primer sequences for the Cyt *b* gene region did not yield quality sequences, but the quality of reverse sequences were deemed sufficient for further analysis. An alignment of 93 reverse sequences was then used for subsequent analysis. Following the trimming of low quality ends, the final length of the trimmed assemblies of the Cyt *b* gene region obtained was 405bp.

A sequence length of 243-446bp was amplified from each giraffe sample using the primer pair Giraffe-DL Fw and Giraffe-DL Rev for the D-loop region. Both the forward and reverse primers produced readable sequences. An alignment of 69 forward and reverse sequences was subsequently used for analysis. Following the trimming of low quality ends, the final length of the trimmed assemblies of the D-loop region obtained from Geneious Pro v4.7.4 was 267bp.

3.3 Genetic diversity

3.3.1 Genetic diversity within populations

3.3.1.1 Diversity at the Cyt *b* region

3.3.1.1.1 Cyt *b* haplotype frequencies

All giraffe sampled for the current study displayed one of two distinct haplotypes at the Cyt *b* region (Table 3.4). A total of 72 individuals from the Free State Province shared haplotype 1. A second haplotype was found in 19 individuals, comprising 12 individuals from the Northern Cape Province and seven giraffe from the Free State

Province. The two haplotypes observed have been submitted to the GenBank database (MH033837 and MH033838), and are also presented in Appendix A.

When the results from the current study were combined with all available giraffe Cyt *b* sequences available from GenBank, a total of 16 haplotypes were observed. Of all the populations that amplified for the Cyt *b* gene, 13 populations displayed Haplotype 1 only (MNR 2, PNR 2, PWR 1, PWR 2, PWR 3, PWR 5, PWR 6, PWR 7, PWR 8, PWR 12, PWR 13, PWR 15, and PWR 16), two populations displayed both Haplotype 1 and Haplotype 2 (PWR 4 and PWR 11), and two populations displayed Haplotype 2 only (PWR 14 and PWR 17).

Table 3.4 The 16 haplotypes identified among Free State Province and Northern Cape Province giraffe. The haplotype present within each population, and the number of individuals exhibiting each haplotype is also shown. [FS – Free State; NC – Northern Cape].

Haplotype number	Total number of sequences (240)	Number of Free State sequences (79)	Number of Northern Cape sequences (12)	Number of GenBank sequences (149)
Haplotype 1 (MH033837)	93	72	-	21
Haplotype 2 (MH033838)	22	7	12	3
Haplotype 3	1	-	-	1
Haplotype 4	1	-	-	1
Haplotype 5	2	-	-	2
Haplotype 6	1	-	-	1
Haplotype 7	4	-	-	4
Haplotype 8	1	-	-	1
Haplotype 9	78	-	-	78
Haplotype 10	1	-	-	1
Haplotype 11	10	-	-	10
Haplotype 12	14	-	-	14
Haplotype 13	1	-	-	1
Haplotype 14	9	-	-	9
Haplotype 15	1	-	-	1
Haplotype 16	1	-	-	1

3.3.1.1.2 Cyt *b* haplotype and nucleotide diversities

Population PWR 4 displayed the highest level of haplotype diversity, with a value of 1.000, with a value of 0.252 observed in population PWR 11 (Table 3.5). Population PWR 4 also showed the highest levels of nucleotide diversity (π = 0.2500), with population PWR 11 having the second highest level of diversity (π = 0.0072). All of the other populations showed a lack of diversity.

The pooled population consisting of all of the Free State populations combined displayed a haplotype diversity of 0.167 and a nucleotide diversity of 0.0004; compared to values of 0.000-1.000 and 0.0000-0.2500 within individual populations. The pooled population consisting of only the small private Free State group had a haplotype diversity of 0.138 and the nucleotide diversity was 0.0003. This compares to values of 0.000-1.000 and 0.0000-0.2500 in individual small populations.

Table 3.5 Haplotype frequencies within populations based on the Cyt *b* gene region. Values in bold represent the only values greater than 0. Nucleotide diversity values are rounded to 5 decimal places to accommodate the small magnitude of differences among populations.

Population	Sample size (n)	# of haplotypes (h)	Haplotype diversity (H_d)	Nucleotide diversity (π)
MNR 2	2	1	0.000	0.0000
PNR 2	12	1	0.000	0.0000
PWR 1	1	-	-	-
PWR 2	3	1	0.000	0.0000
PWR 3	2	1	0.000	0.0000
PWR 4	2	2	**1.000**	**0.2500**
PWR 5	2	1	0.000	0.0000
PWR 6	7	1	0.000	0.0000
PWR 7	1	-	-	-
PWR 8	3	1	0.000	0.0000
PWR 11	35	2	**0.252**	**0.0072**
PWR 12	2	1	0.000	0.0000
PWR 13	1	-	-	-
PWR 14	1	-	-	-
PWR 15	1	-	-	-
PWR 16	2	1	0.000	0.0000
PWR 17	12	1	0.000	0.0000
Small Private Free State populations (SPFSp)	28	2	**0.138**	**0.0003**
All Free State populations (AFSp)	77	2	**0.167**	**0.0004**

3.3.1.2 Diversity at the D-loop region

3.3.1.2.1 D-loop haplotype frequencies

The giraffe sampled for the present study displayed one of ten distinct haplotypes at the D-loop region (Table 3.6). A total of 14 individuals from the Free State Province shared the first haplotype. A second haplotype was found in a single giraffe from this province. A total of 24 individuals shared a third haplotype, comprising of 21 individuals

from the Free State Province and three individuals from the Northern Cape Province. A fourth haplotype was found in a single giraffe from the Free State Province. A fifth haplotype was found in four individuals, comprising of two individuals from the Free State Province and two individuals from the Northern Cape Province. A total of 11 individuals from the Free State Province shared a sixth haplotype. A seventh haplotype was found in four individuals from the Free State Province. A total of six individuals shared an eighth haplotype, comprising of five individuals from the Free State Province and one individual from the Northern Cape Province. A ninth haplotype was found in four individuals from the Free State Province. A single individual from the Northern Cape Province displayed the tenth haplotype. The ten haplotypes observed have been submitted to the GenBank database (MH033839, MH033840, MH033841, MH033842, MH033843, MH033844, MH033845, MH033846, MH033847, and MH033848), and is also presented in Appendix A.

When the results from the present study were combined with all available giraffe D-loop sequences from GenBank, a total of 34 haplotypes were observed. Of all the populations that amplified for the D-loop region, six populations displayed Haplotype 1 (PNR 2, PWR 3, PWR 6, PWR 9, PWR 11, PWR 13), one population displayed Haplotype 2 (PWR 1), 11 populations displayed Haplotype 3 (MNR 1, PNR 2, PWR 3, PWR 5, PWR 6, PWR 8, PWR 9, PWR 10, PWR 11, PWR 16, and PWR 17), one population displayed Haplotype 4 (PWR 4), three populations displayed Haplotype 5 (PWR 4, PWR 14, and PWR17), two populations displayed Haplotype 6 (MNR 2 and PWR 11), three populations displayed Haplotype 7 (PWR 2, PWR 11, and PWR 15), two populations displayed Haplotype 8 (PWR 11 and PWR 17), one population displayed Haplotype 9 (PWR 11), and one population displayed Haplotype 10 (PWR 17).

Table 3.6 The 34 haplotypes identified among Free State Province and Northern Cape Province giraffe. The haplotype present within each population, and the number of individuals exhibiting each haplotype is also shown. [FS – Free State; NC – Northern Cape].

Haplotype number	Total number of sequences (247)	Number of Free State sequences (63)	Number of Northern Cape sequences (7)	Number of GenBank sequences (177)
Haplotype 1 (MH033839)	58	14	-	44
Haplotype 2 (MH033840)	1	1	-	-
Haplotype 3 (MH033841)	27	21	3	3
Haplotype 4 (MH033842)	7	1	-	6
Haplotype 5 (MH033843)	4	2	2	-
Haplotype 6 (MH033844)	13	11	-	2
Haplotype 7 (MH033845)	7	4	-	3
Haplotype 8 (MH033846)	13	5	1	7
Haplotype 9 (MH033847)	11	4	-	7
Haplotype 10 (MH033848)	1	-	1	-
Haplotype 11	5	-	-	5
Haplotype 12	11	-	-	11
Haplotype 13	1	-	-	1
Haplotype 14	35	-	-	35
Haplotype 15	2	-	-	2
Haplotype 16	7	-	-	7
Haplotype 17	1	-	-	1
Haplotype 18	1	-	-	1
Haplotype 19	1	-	-	1
Haplotype 20	9	-	-	9
Haplotype 21	8	-	-	8
Haplotype 22	6	-	-	6

Haplotype 23	3	-	-	3
Haplotype 24	1	-	-	1
Haplotype 25	1	-	-	1
Haplotype 26	1	-	-	1
Haplotype 27	1	-	-	1
Haplotype 28	1	-	-	1
Haplotype 29	1	-	-	1
Haplotype 30	2	-	-	2
Haplotype 31	1	-	-	1
Haplotype 32	3	-	-	3
Haplotype 33	2	-	-	2
Haplotype 34	1	-	-	1

3.3.1.2.2 D-loop haplotype and nucleotide diversities

Populations PWR 3 and PWR 4 showed the highest haplotype diversity, with values of 1.000 each. The only other populations to display polymorphism were, in descending order of diversity, populations PWR 11, PWR 17, PWR 9, PNR 2, and PWR 6 (Table 3.7).

Nucleotide diversity (π) values from the D-loop region showed that the PWR 17 population (π = 0.0276) possessed the highest levels of nucleotide diversity, with population PWR 11 having the second highest level (π = 0.0163). The only other populations showing any nucleotide diversity were populations PWR 4 (π = 0.0145), PNR 2 (π = 0.0120), PWR 3 (π = 0.0036), PWR 9 (π = 0.0018) and PWR 6 (π = 0.0015). All of the other populations showed no nucleotide diversity (Table 3.8).

The pooled population consisting of all of the Free State populations combined displayed a haplotype diversity of 0.805 and the nucleotide diversity was 0.0277; compared to values of 0.000-1.000 and 0.0000-0.0276 within individual populations. The pooled population consisting of only the small private Free State group had a haplotype diversity of 0.646 and the nucleotide diversity was 0.0203. This compares to values of 0.000-1.000 and 0.0000-0.0163 in individual small populations.

Table 3.7 Haplotype frequencies within populations based on the D-loop region. Values in bold represent the only values greater than 0. Nucleotide diversity values are rounded to 5 decimal places to accommodate the small magnitude of differences among populations.

Population	Sample size (n)	# of haplotypes (h)	Haplotype diversity (Hd)	Nucleotide diversity (π)
MNR 1	1	-	-	-
MNR 2	3	1	0.000	0.0000
PNR 2	13	2	**0.462**	**0.0120**
PWR 1	1	-	-	-
PWR 2	2	1	0.000	0.0000
PWR 3	2	2	**1.000**	**0.0036**
PWR 4	2	2	**1.000**	**0.0145**
PWR 5	1	-	-	-
PWR 6	5	2	**0.400**	**0.0015**
PWR 8	3	1	0.000	0.0000
PWR 9	4	2	**0.500**	**0.0018**
PWR 10	1	-	-	-
PWR 11	20	6	**0.768**	**0.0163**
PWR 13	1	-	-	-
PWR 14	1	-	-	-
PWR 15	1	-	-	-
PWR 16	2	1	0.000	0.0000
PWR 17	7	3	**0.762**	**0.0276**
Small Private Free State populations (SPFSp)	26	6	**0.646**	**0.0203**
All Free State populations (AFSp)	62	9	**0.805**	**0.0277**

3.3.1.3 Overall diversity at both the Cyt *b* and D-loop region

As indicated in Table 3.8, only a few populations had meaningful values. The averages were calculated for the Cyt *b* region and the D-loop region. PWR 4 has the highest haplotype (H_d = 1.000) and nucleotide (π = 0.1323) diversities, where PWR 6 has the lowest haplotype (H_d = 0.200) and nucleotide (π = 0.0007) diversities.

All of the Free State populations combined had a haplotype diversity of 0.486, where the nucleotide diversity was 0.0141. The small private Free State group had a haplotype diversity of 0.392, where the nucleotide diversity was 0.0103.

Table 3.8 An overview of both genes' haplotype and nucleotide diversities. Values in bold represent the only values greater than 0. Nucleotide diversity values are rounded to 5 decimal places to accommodate the small magnitude of differences among populations.

Population	Haplotype diversity (H_d)	Nucleotide diversity (π)
MNR 1	-	-
MNR 2	0.000	0.0000
PNR 1	-	-
PNR 2	**0.231**	**0.0060**
PWR 1	0.000	0.0000
PWR 2	0.000	0.0000
PWR 3	**0.500**	**0.0018**
PWR 4	**1.000**	**0.1323**
PWR 5	0.000	0.0000
PWR 6	**0.200**	**0.0007**
PWR 7	-	-
PWR 8	0.000	0.0000
PWR 9	**0.250**	**0.0009**
PWR 10	-	-
PWR 11	**0.510**	**0.0118**
PWR 12	0.000	0.0000
PWR 13	-	-
PWR 14	-	-
PWR 15	-	-
PWR 16	0.000	0.0000
PWR 17	**0.381**	**0.0138**
Small Private Free State populations (SPFSp)	**0.392**	**0.0103**
All Free State populations (AFSp)	**0.486**	**0.0141**

3.4 Genetic differentiation

The F_{ST} values and associated p-values among population pairs are listed in Table 3.9 for the Cyt *b* gene and Table 3.10 for the D-loop region. The values calculated for the average number of nucleotide differences between populations for the Cyt *b* gene are indicated in Table 3.11, whereas Table 3.12 indicates the average number of nucleotide differences between populations for the D-loop region. Tables 3.13 and 3.14 shows the average number of nucleotide substitutions per site between populations for the Cyt *b* gene and D-loop region respectively. The values calculated for the number of net nucleotide substitutions per site between populations for the Cyt *b* gene and D-loop region are indicated in Tables 3.15 and 3.16.

The same trend was evident from all measures of genetic differentiation used, and only results from F_{ST} will be detailed. For the Cyt *b* gene, F_{ST} values showed more similarity between giraffe from the Free State Province and *G. c. angolensis* (F_{ST} = 0), compared to Free State versus *G. c. giraffa* (F_{ST} = 0.969). The later value is comparable to F_{ST} between the Free State and Northern Cape populations, F_{ST}=0.849. All other subspecies showed more differentiation from the Free State population with F_{ST} values of 0.981-0.992.

For the D-loop region, the Free State population also showed more identity with *G. c. angolensis* compared to *G. c. giraffa*, with F_{ST} values of 0.068 and 0.345 respectively. For comparisons between the Free State population and the remaining subspecies, F_{ST} values of 0.340-0.663 were observed. Results from the D-loop region showed no differentiation (F_{ST} = 0) between giraffe from the Free State and Northern Cape Provinces.

Table 3.9 F_{ST} values among giraffe populations based on the Cyt *b* gene region, with corresponding p-values. Orange Cells – Free State populations; Yellow Cells – Northern Cape population; Blue Cells – Giraffe subspecies.

	Free State	**Northern Cape**	***G. c. angolensis***	***G. c. antiquorum***	***G. c. giraffa***	***G. c. peralta***	***G. c. reticulata***	***G. c. rothschildi***	***G. c. thornicrofti***
Free State	-								
Northern Cape	0.849 (0)	-							
G. c. angolensis	0 (0.991)	0.840 (0)	-						
G. c. antiquorum	0.983 (0)	0.968 (0)	0.971 (0)	-					
G. c. giraffa	0.969 (0)	0.962 (0)	0.958 (0)	0.962 (0)	-				
G. c. peralta	0.992 (0)	1.000 (0)	0.992 (0)	0.890 (0)	0.978 (0)	-			
G. c. reticulata	0.988 (0.991)	1.000 (0.991)	0.985 (0.991)	0.698 (0.991)	0.969 (0.991)	1.000 (0.991)	-		
G. c. rothschildi	0.991 (0)	1.000 (0)	0.991 (0)	0.827 (0)	0.975 (0)	1.000 (0)	1.000 (0.721)	-	
G. c. thornicrofti	0.987 (0)	1.000 (0)	0.990 (0)	0.984 (0)	0.094 (0)	1.000 (0)	1.000 (0.180)	1.000 (0)	-
G. c. tippelskirchi	0.981 (0)	0.990 (0)	0.975 (0)	0.945 (0)	0.042 (0.243)	0.995 (0)	0.981 (0.099)	0.993 (0)	0.268 (0.297)

Table 3.10 F_{ST} values among giraffe populations based on the D-loop region, with corresponding p-values. Orange Cells – Free State populations; Yellow Cells– Northern Cape population; Blue Cells – Giraffe subspecies.

	Free State	**Northern Cape**	***G. c. angolensis***	***G. c. antiquorum***	***G. c. camelopardalis***	***G c. giraffa***	***G. c. peralta***	***G. c. reticulata***	***G. c. rothschildi***	***G. c. thornicrofti***
Free State	-									
Northern Cape	0 (0.342)	-								
G. c. angolensis	0.068 (0.018)	0 (0.514)	-							
G. c. antiquorum	0.567 (0)	0.592 (0)	0.564 (0)	-						
G. c. camelopardalis	0.578 (0)	0.574 (0)	0.575 (0)	0.180 (0.180)	-					
G c. giraffa	0.345 (0)	0.692 (0)	0.587 (0)	0.921 (0)	0.909 (0)	-				
G. c. peralta	0.663 (0)	0.803 (0)	0.675 (0)	0.848 (0)	0.751 (0)	0.945 (0)	-			
G. c. reticulata	0.615 (0)	0.611 (0)	0.617 (0)	0.567 (0.009)	0.448 (0)	0.901 (0)	0.737 (0)	-		
G. c. rothschildi	0.625 (0)	0.677 (0)	0.632 (0)	0.685 (0)	0.482 (0)	0.904 (0)	0.802 (0)	0.585 (0)	-	
G. c. thornicrofti	0.537 (0)	0.838 (0)	0.643 (0)	0.981 (0)	0.966 (0)	0.914 (0)	0.994 (0)	0.944 (0)	0.950 (0)	-
G. c. tippelskirchi	0.340 (0)	0.438 (0)	0.445 (0)	0.811 (0)	0.767 (0)	0.818 (0)	0.932 (0)	0.752 (0)	0.819 (0)	0.907 (0)

Table 3.11 The average number of nucleotide differences between giraffe populations based on the Cyt *b* gene region. Values are rounded to 1 decimal place to accommodate the small magnitude of differences among populations. Orange Cells – Free State populations; Yellow Cells– Northern Cape population; Blue Cells – Giraffe subspecies.

	Free State	**Northern Cape**	***G. c. angolensis***	***G. c. antiquorum***	***G. c. giraffa***	***G. c. peralta***	***G. c. reticulata***	***G. c. rothschildi***	***G. c. thornicrofti***
Free State	-								
Northern Cape	0.9	-							
G. c. angolensis	0.2	0.9	-						
G. c. antiquorum	16.0	16.5	16.1	-					
G. c. giraffa	9.2	10.1	9.2	15.7	-				
G. c. peralta	18.1	19.0	18.1	4.5	17.2	-			
G. c. reticulata	14.1	15.0	14.1	4.5	15.2	4.0	-		
G. c. rothschildi	17.1	18.0	17.1	3.5	16.2	3.0	3.0	-	
G. c. thornicrofti	9.1	10.0	9.1	15.5	0.3	17.0	15.0	16.0	-
G. c. tippelskirchi	9.2	10.1	9.3	15.6	0.4	17.1	15.1	16.1	0.1

Table 3.12 The average number of nucleotide differences between giraffe populations based on the D-loop region. Values are rounded to 1 decimal place to accommodate the small magnitude of differences among populations. Orange Cells – Free State populations; Yellow Cells– Northern Cape population; Blue Cells – Giraffe subspecies.

	Free State	**Northern Cape**	***G. c. angolensis***	***G. c. antiquorum***	***G. c. camelopardalis***	***G. c. giraffa***	***G. c. peralta***	***G. c. reticulata***	***G. c. rothschildi***	***G. c. thornicrofti***
Free State	-									
Northern Cape	7.3	-								
G. c. angolensis	7.7	7.1	-							
G. c. antiquorum	14.4	13.9	14.2	-						
G. c. camelopardalis	15.9	15.7	15.7	5.1	-					
G. c. giraffa	6.8	8.2	9.2	17.5	18.3	-				
G. c. peralta	15.9	14.9	15.2	6.5	7.4	19.1	-			
G. c. reticulata	17.3	17.4	17.4	10.7	10.4	18.8	9.0	-		
G. c. rothschildi	16.8	16.2	15.6	11.7	9.0	17.2	10.0	11.7	-	
G. c. thornicrofti	8.5	8.6	9.4	16.0	18.4	7.1	16.8	18.3	18.0	-
G. c. tippelskirchi	8.2	8.4	9.6	13.9	15.5	6.9	16.1	17.0	18.3	5.3

Table 3.13 The average number of nucleotide substitutions per site between giraffe populations based on the Cyt *b* gene region. Values are rounded to 3 decimal places to accommodate the small magnitude of differences among populations. Orange Cells – Free State populations; Yellow Cells– Northern Cape population; Blue Cells – Giraffe subspecies.

	Free State	**Northern Cape**	***G. c. angolensis***	***G. c. antiquorum***	***G. c. giraffa***	***G. c. peralta***	***G. c. reticulata***	***G. c. rothschildi***	***G. c. thornicrofti***
Free State	-								
Northern Cape	0.002	-							
G. c. angolensis	0.001	0.002	-						
G. c. antiquorum	0.040	0.041	0.040	-					
G. c. giraffa	0.023	0.025	0.023	0.039	-				
G. c. peralta	0.045	0.047	0.045	0.011	0.042	-			
G. c. reticulata	0.035	0.037	0.035	0.011	0.038	0.010	-		
G. c. rothschildi	0.042	0.044	0.042	0.009	0.040	0.007	0.007	-	
G. c. thornicrofti	0.023	0.025	0.023	0.038	0.001	0.042	0.037	0.040	-
G. c. tippelskirchi	0.023	0.025	0.023	0.039	0.001	0.042	0.037	0.040	0.000

Table 3.14 The average number of nucleotide substitutions per site between giraffe populations based on the D-loop region. Values are rounded to 3 decimal places to accommodate the small magnitude of differences among populations. Orange Cells – Free State populations; Yellow Cells– Northern Cape population; Blue Cells – Giraffe subspecies.

	Free State	**Northern Cape**	***G. c. angolensis***	***G. c. antiquorum***	***G. c. camelopardalis***	***G. c. giraffa***	***G. c. peralta***	***G. c. reticulata***	***G. c. rothschildi***	***G. c. thornicrofti***
Free State	-									
Northern Cape	0.028	-								
G. c. angolensis	0.029	0.027	-							
G. c. antiquorum	0.054	0.052	0.053	-						
G. c. camelopardalis	0.060	0.059	0.059	0.019	-					
G. c. giraffa	0.026	0.031	0.034	0.066	0.069	-				
G. c. peralta	0.060	0.056	0.057	0.024	0.028	0.072	-			
G. c. reticulata	0.065	0.066	0.066	0.040	0.039	0.071	0.034	-		
G. c. rothschildi	0.063	0.061	0.059	0.044	0.034	0.065	0.037	0.044	-	
G. c. thornicrofti	0.032	0.032	0.035	0.060	0.069	0.027	0.063	0.069	0.067	-
G. c. tippelskirchi	0.031	0.032	0.036	0.052	0.058	0.026	0.061	0.064	0.069	0.020

Table 3.15 The number of net nucleotide substitutions per site between giraffe populations based on the Cyt *b* gene region. Values are rounded to 4 decimal places to accommodate the small magnitude of differences among populations. Orange Cells – Free State populations; Yellow Cells– Northern Cape population; Blue Cells – Giraffe subspecies.

	Free State	**Northern Cape**	***G. c. angolensis***	***G. c. antiquorum***	***G. c. giraffa***	***G. c. peralta***	***G. c. reticulata***	***G. c. rothschildi***	***G. c. thornicrofti***
Free State	-								
Northern Cape	0.0020	-							
G. c. angolensis	0.0000	0.0019	-						
G. c. antiquorum	0.0377	0.0391	0.0377	-					
G. c. giraffa	0.0219	0.0244	0.0219	0.0364	-				
G. c. peralta	0.0445	0.0469	0.0445	0.0094	0.0419	-			
G. c. reticulata	0.0346	0.0370	0.0346	0.0094	0.0369	0.0099	-		
G. c. rothschildi	0.0420	0.0444	0.0420	0.0070	0.0394	0.0074	0.0074	-	
G. c. thornicrofti	0.0222	0.0247	0.0223	0.0366	0.0001	0.0420	0.0370	0.0395	-
G. c. tippelskirchi	0.0222	0.0247	0.0223	0.0366	0.0001	0.0420	0.0370	0.0395	0.0000

Table 3.16 The number of net nucleotide substitutions per site between giraffe populations based on the D-loop region. Values are rounded to 4 decimal places to accommodate the small magnitude of differences among populations. Orange Cells – Free State populations; Yellow Cells– Northern Cape population; Blue Cells – Giraffe subspecies.

	Free State	**Northern Cape**	***G. c. angolensis***	***G. c. antiquorum***	***G. c. camelopardalis***	***G. c. giraffa***	***G. c. peralta***	***G. c. reticulata***	***G. c. rothschildi***	***G. c. thornicrofti***
Free State	-									
Northern Cape	-0.0002	-								
G. c. angolensis	0.0020	-0.0003	-							
G. c. antiquorum	0.0347	0.0331	0.0349	-						
G. c. camelopardalis	0.0364	0.0359	0.0365	0.0034	-					
G. c. giraffa	0.0094	0.0147	0.0189	0.0579	0.0568	-				
G. c. peralta	0.0452	0.0417	0.0434	0.0183	0.0167	0.0686	-			
G. c. reticulata	0.0398	0.0404	0.0410	0.0229	0.0164	0.0567	0.0211	-		
G. c. rothschildi	0.0420	0.0400	0.0385	0.0310	0.0159	0.0553	0.0292	0.0250	-	
G. c. thornicrofti	0.0181	0.0185	0.0222	0.0547	0.0584	0.0241	0.0622	0.0568	0.0600	-
G. c. tippelskirchi	0.0127	0.0135	0.0187	0.0425	0.0445	0.0194	0.0557	0.0483	0.0573	0.0156

3.5 Relationships among haplotypes

3.5.1 Phylogenetic analysis

The results for the Cyt *b* region, based on a maximum likelihood (ML) approach, is presented in Figure 3.1, with the results for this gene generated using a Bayesian approach presented in Figure 3.2. Both analyses revealed the same broad tree topology, and only trends from ML are discussed below.

The overall distribution of haplotypes shows three distinct clusters, consisting of the northern and southern groups as well as the outgroup. The northern clade (*G. c. antiquorum*, *G. c. camelopardalis*, *G. c. peralta*, *G. c. reticulata*, *G. c. rothschildi*) is supported by a high bootstrap value of 84%. The southern subspecies (*G. c. angolensis*, *G. c. giraffa*, *G. c. thornicrofti*, and *G. c. tippelskirchi*) also clustered together, but with somewhat lower bootstrap support at 54%. The reference samples from *O. johnstoni* form a well-supported monophyletic cluster with 100% bootstrap support.

Within the southern cluster, giraffe sequenced during the current study cluster with *G. c. angolensis*, with a bootstrap value of 81%. There was no specific pattern to reflect distinction between giraffe from the Free State and Northern Cape provinces. The remaining three subspecies form a clade with 93% bootstrap support.

For the D-loop region, output based on the ML method is presented in Figure 3.3, with results from a Bayesian approach given in Figure 3.4. As with the Cyt *b* region, both approaches yielded the same broad topology, and only trends from ML are discussed below.

Three distinct clusters were observed, corresponding to the northern group, the southern group and the reference group. The clade containing the northern subspecies is supported by a bootstrap value of 75%. Note that one individual of *G. c. rothschildi* grouped outside this otherwise monophyletic northern cluster. The reference material (*O. johnstoni*) form a well-supported, distinct group, as expected.

Within the southern giraffe cluster, the pattern of clustering is less distinct than that obtained using the Cyt *b* region, and with generally low bootstrap support. Giraffe sequenced during the current study clustered with both *G. c. angolensis* and *G. c. giraffa*. As for Cyt *b*, there was no specific pattern to reflect distinction between giraffe from the Free State and Northern Cape provinces.

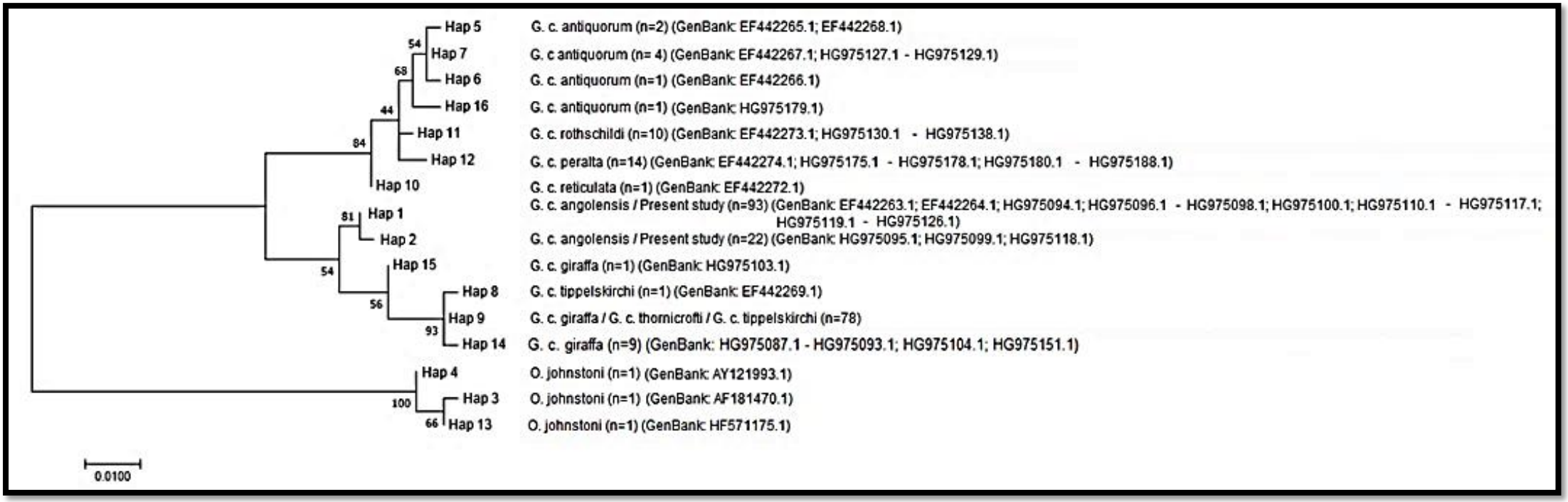

Figure 3.1 Molecular phylogenetic analysis of giraffe Cyt *b* sequences using a Maximum Likelihood approach. The percentage of trees in which the associated taxa are clustered together are shown next to the branches, based on 1 000 bootstrap replications. This tree is drawn to scale, with branch lengths measured in the number of substitutions per site. The analysis was constructed using 16 haplotypes. All gaps and missing data were eliminated. In the final set, there was 405bp positions. Evolutionary analysis was conducted using Mega v7.0.26.

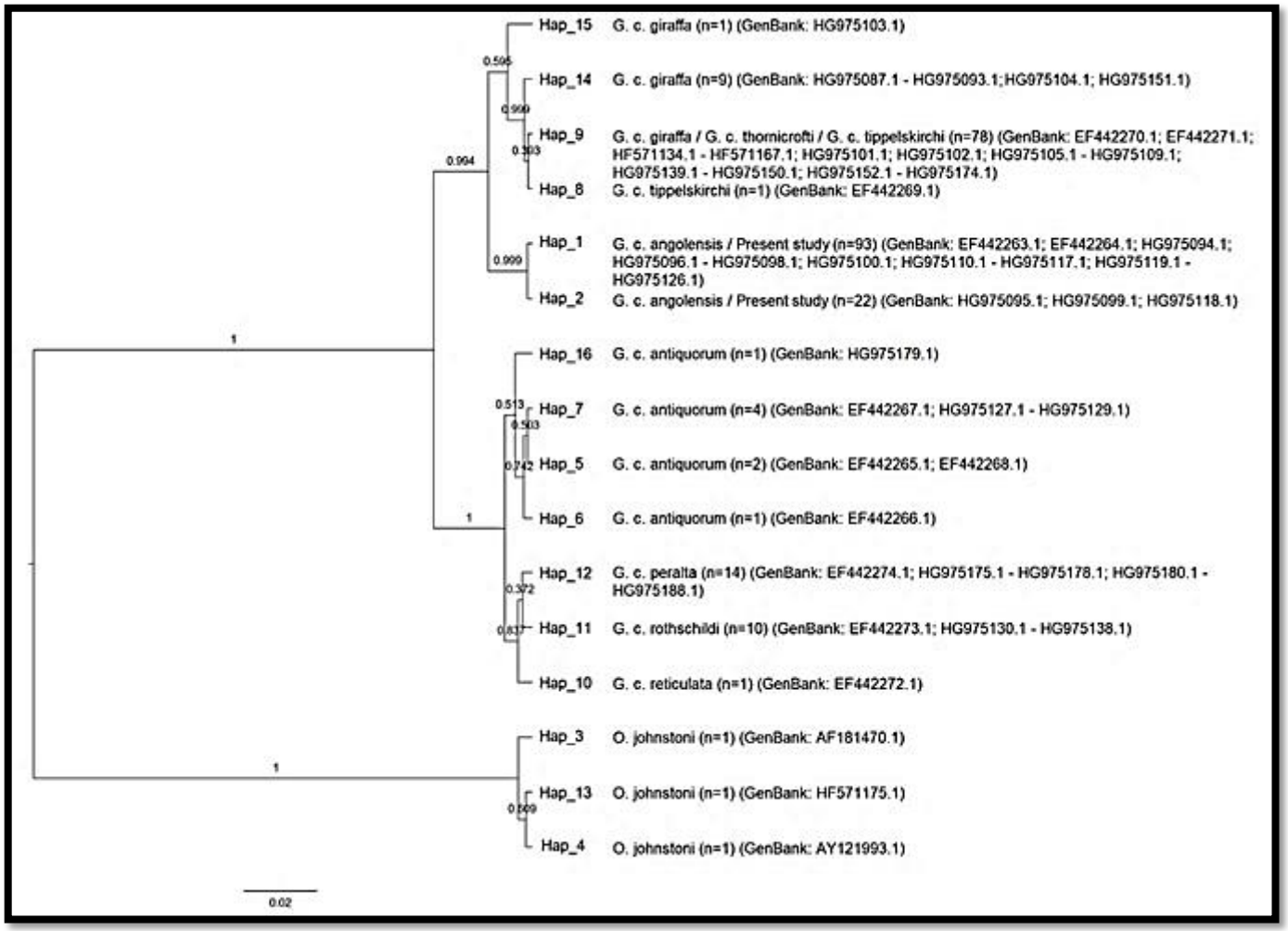

Figure 3.2 Phylogenetic tree generated using BEAST v1.7, based on a 405bp fragment of the Cyt *b* gene region, and 16 giraffe haplotypes. Published *Okapia johnstoni* sequences were used as an outgroup. The posterior probabilities for branching points are provided next to every branch. The number of individuals is given in brackets, next to each subspecies name, with GenBank accession numbers for reference sequences used.

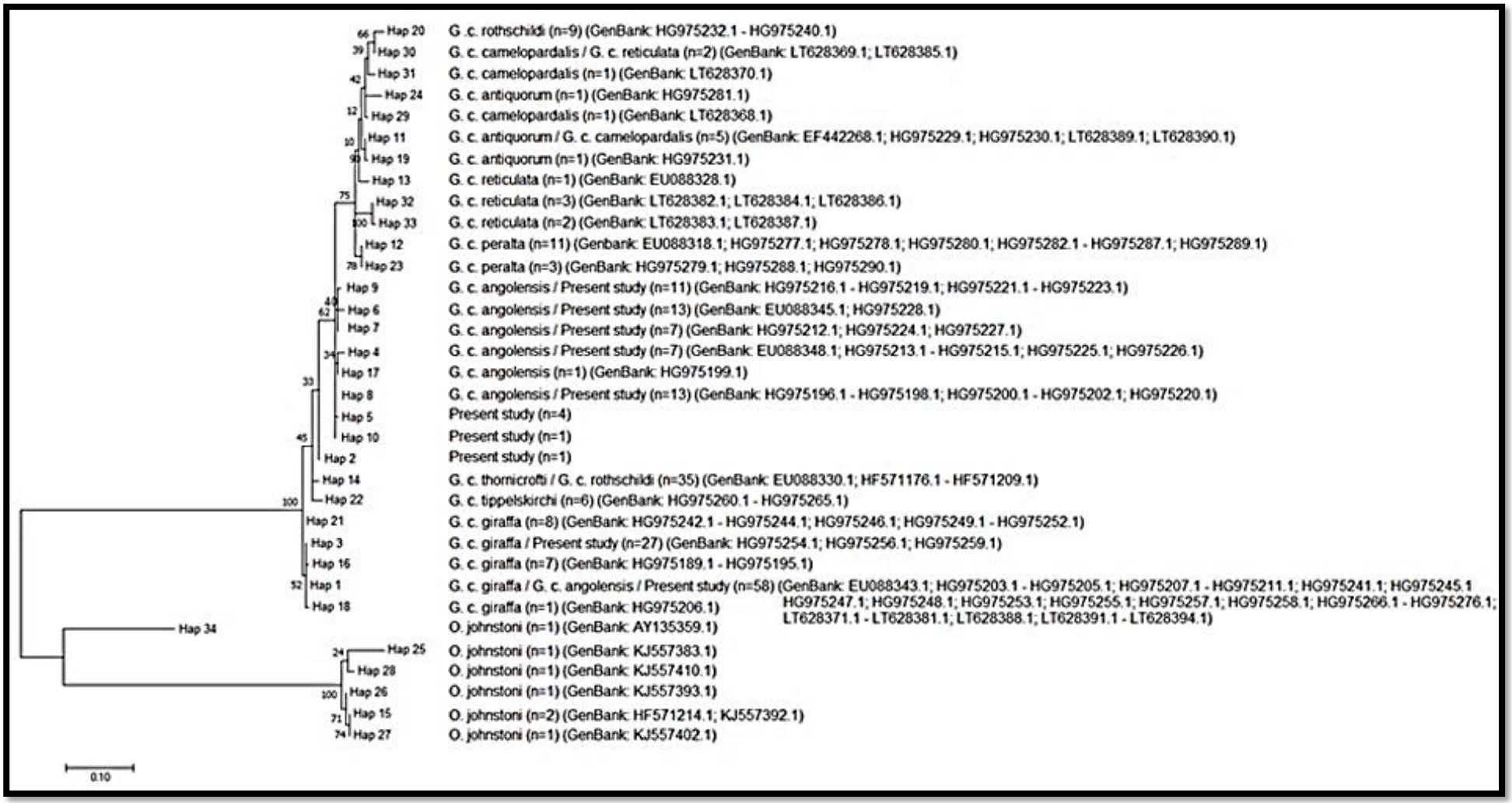

Figure 3.3 Molecular phylogenetic analysis of giraffe D-loop sequences using a Maximum Likelihood approach. The percentage of trees in which the associated taxa are clustered together are shown next to the branches, based on 1 000 bootstrap replications. This tree is drawn to scale, with branch lengths measured in the number of substitutions per site. The analysis was constructed using 34 haplotypes. All gaps and missing data were eliminated. In the final set, there was 267bp positions. Evolutionary analysis was conducted using Mega v7.0.26.

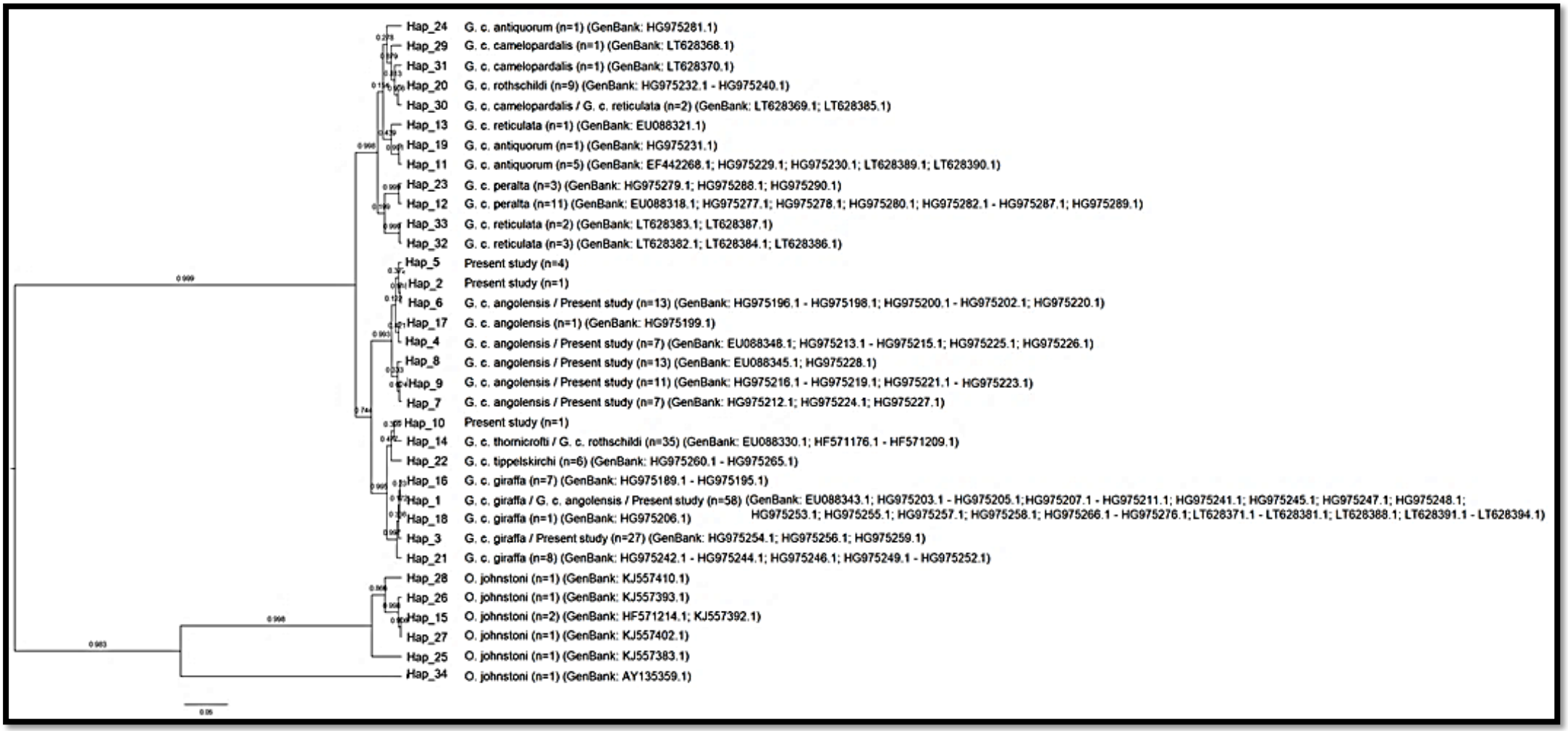

Figure 3.4 Phylogenetic tree generated using BEAST v1.7, based on a 267bp fragment of the D-loop region, and 34 giraffe haplotypes. Published *Okapia johnstoni* sequences were used as an outgroup. The posterior probabilities for branching points are provided next to every branch. The number of individuals is given in brackets, next to each subspecies name, with GenBank accession numbers for reference sequences used.

3.5.2 Haplotype Networks

Figure 3.5 depicts the network based on Cyt *b* haplotypes, with the D-loop based haplotype network shown in Figure 3.6. Overall, the samples found in both the Free State and Northern Cape provinces show substantial levels of identity.

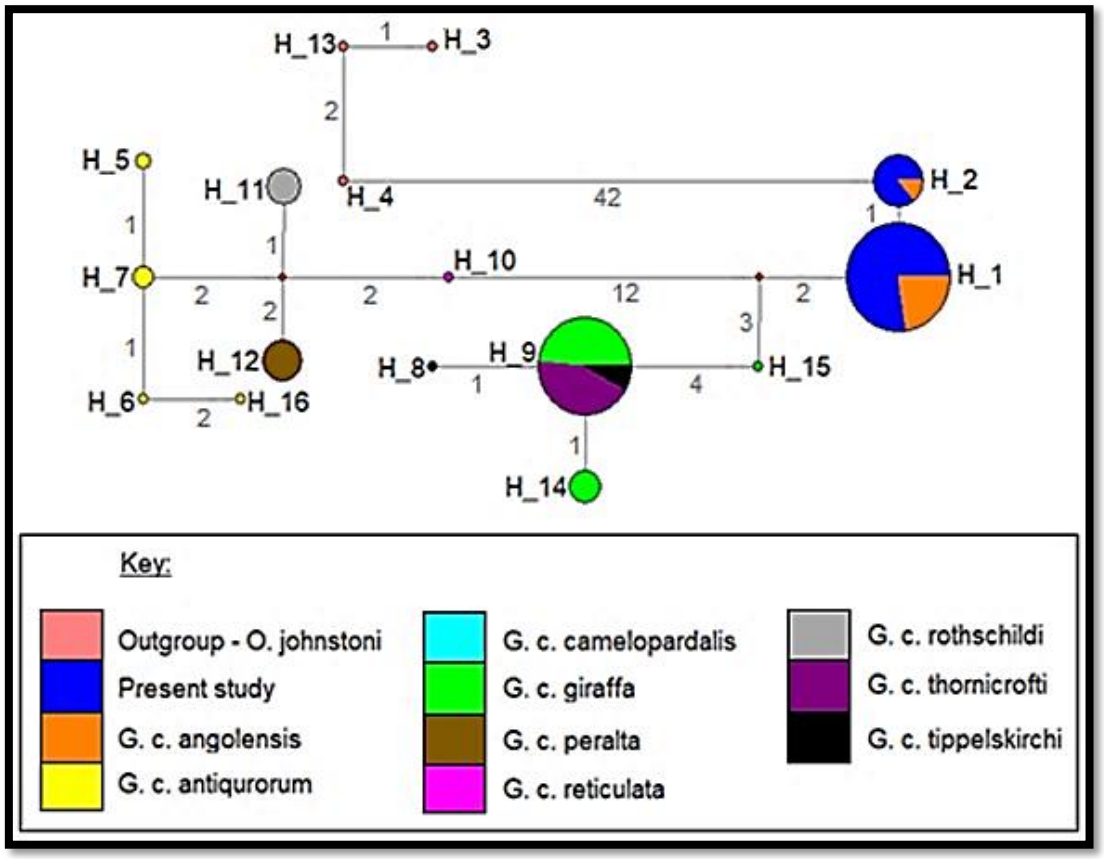

Figure 3.5 A visualisation of the phylogenetic network based on the Cyt *b* sequences for giraffe. The numbering between the nodes is an indication of the number of mutational steps between each haplotype observed. The size of the circles is indicative of the number of individuals associated with that specific haplotype.

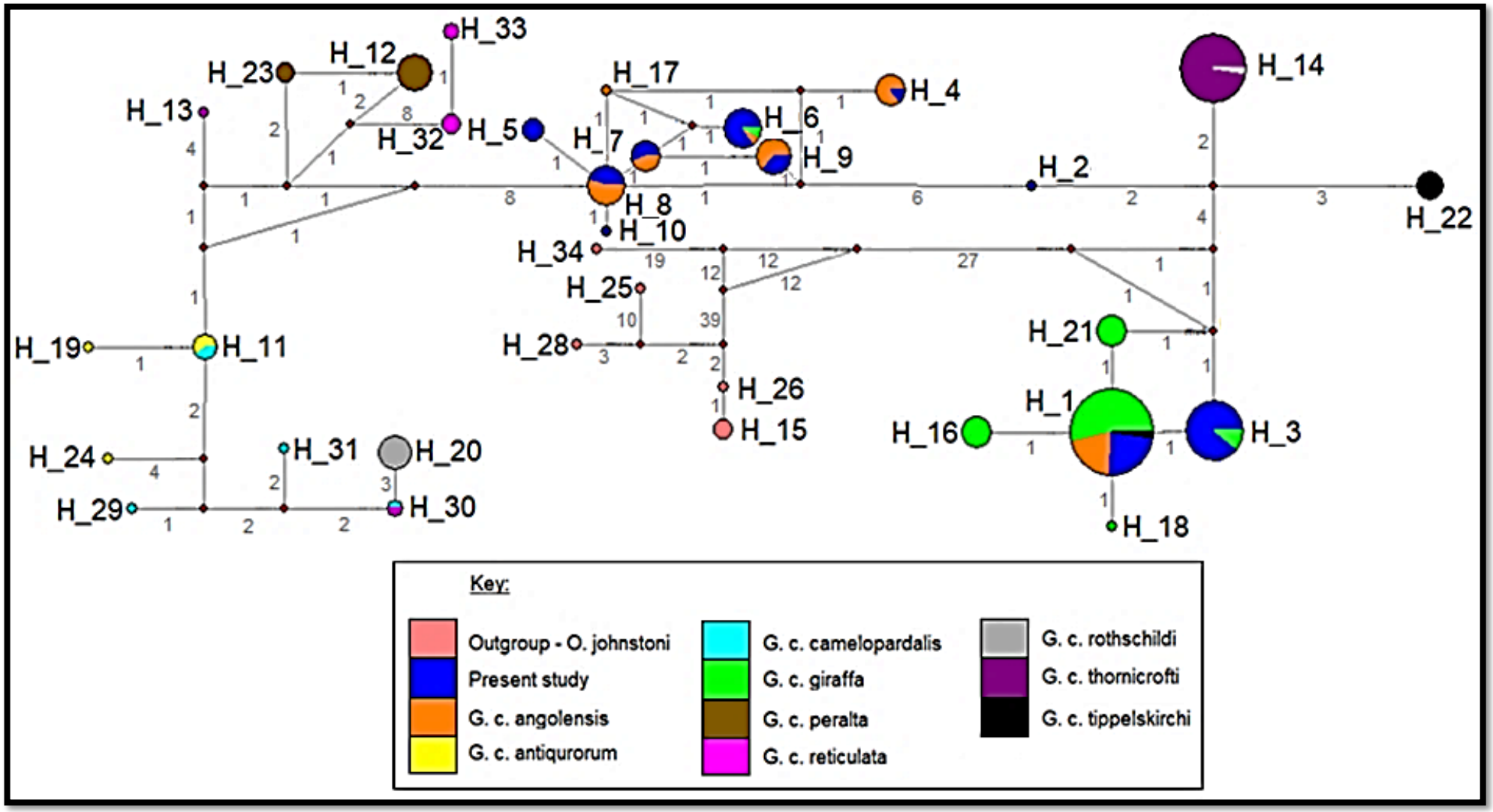

Figure 3.6 A visualisation of the phylogenetic network based on the D-loop sequences for giraffe. The numbering between the nodes is an indication of the number of mutational steps between each haplotype observed. The size of the circles is indicative of the number of individuals associated with that specific haplotype.

In Figure 3.5, the Cyt *b* region haplotypes generated during the current study, form part of a closely related *G. c. angolensis* group. These same two haplotypes also occurred in the *G. c. angolensis* reference material. These two haplotypes (Haplotype 1 and Haplotype 2) are separated by a single mutational step. The next closest haplotype (Haplotype 15), was separated from Haplotypes 1 & 2 by five mutational events. The reference sequences from *O. johnstoni* were separated from the *G. camelopardalis* clusters by 42 or more steps. The northern and southern groups of subspecies cluster as distinct separate sets, with 10 or more mutational steps separating these groups.

The 10 D-loop haplotypes generated from this study clustered with two distinct groups of *G. camelopardalis* (Figure 3.6). Haplotypes 1, 2, and 3 form part of the *G. c. giraffa* group, whereas Haplotypes 4, 5, 6, 7, 8, 9, and 10 form part of the *G. c. angolensis* group. These two groups are separated by 15 or more mutational steps. The reference haplotypes from *O. johnstoni* were separated from the *G. camelopardalis* clusters by 83 or more steps. As for Cyt *b*, the northern and southern groups cluster separately, with 10 or more mutational steps separating the groups.

The implications of the results obtained on genetic diversity and differentiation are discussed in the following chapter.

Chapter 4.

Discussion and Conclusion

In concluding this study, it is appropriate to return to the original specific aims of the study and to determine to what extent these have been met. The first aim of this study was to quantify the levels of genetic diversity within individual giraffe populations, in order to determine the effect of isolation and fragmentation. The second aim was to determine the genetic structure of *Giraffa camelopardalis* in the Free State; i.e. determine the relationships among populations and also the relationship between these South African populations and other populations and subspecies of giraffe.

4.1 Genetic diversity within populations

The results of the present study confirmed our expectations, with more diversity present in the larger populations compared to the smaller populations.

Populations PNR 2 and PWR 11 were considered as the control populations for this study, as they had a larger population size in comparison to the other populations sampled and/or well-structured founder populations. Population PNR 2 is a Provincial protected area in the Free State Province. The population size consisted of 21 individuals at the time of sampling, and the sample size comprised of 17 individuals. The management history of this reserve is well-known. The giraffe were translocated from the Limpopo SPCA, Hans Merensky Reserve in the Limpopo Province, as well as from the Weenen area in Kwa-Zulu Natal. Population PWR 11 is from a private wildlife farm in the Free State Province, which also had a large population size. The population consisted of 37 individuals at the time of sampling, and the sample size comprised of 35 giraffe. The exact management history of the farm is unknown; however, it is known that giraffe were bought over several years from various localities. From the results obtained, it can be inferred that the translocation of individuals from various localities into both populations, contributed towards high levels of diversity within these populations.

The general magnitude of values from the present study follow the same trend as those found by Brown *et al*. (2007). These authors found generally low haplotype and nucleotide diversity from giraffe control region sequences. Bock *et al*. (2014) also found relatively low haplotype and nucleotide diversities in the various subspecies,

however, higher haplotype and nucleotide diversities were found in *G. c. antiquorum* and *G. c. reticulata*.

Looking at the smaller populations, levels of diversity were relatively low in many populations. There were, however, a few small populations with higher diversity levels than the others. This included PWR 3, PWR 4, PWR 6, and PWR 9. In general, not many Private Wildlife Reserves keep more than five giraffe in a population. Only six of the 16 Free State Private Wildlife Reserves harboured five or more giraffe. Furthermore, in most cases the giraffe populations were founded using animals bought from a single source population and all animals were bought at the same time. Population genetics theory predicts that this often leads to there being little to no genetic diversity in the population, which explain the results from the current study.

The role of sample size should be carefully considered in the context of the current results. Ideally, it is not advisable to quantify or compare levels of diversity for very small sample sizes. In line with this, in the cases where there was only a single giraffe, genetic diversity could not be calculated at all. However, if a population is small in size, and almost all or all individuals were sampled, the values must indeed be considered representative for that population.

Of the small populations which had higher levels of diversity, PWR 3 and PWR 4 had unknown management histories. The population size of PWR 3 at the time of sampling consisted of more than 10 individuals, with 10 individuals sampled for the current study (with not all sequenced successfully). Similarly, population PWR 4 had a census population size of two giraffe, and both these giraffe were sampled. Population PWR 6 consisted of four giraffe, which were translocated from a location near Bloemfontein, and another two giraffe were translocated from locality PWR 3. The owners of population PWR 9 originally bought giraffe in 2002, which had gone on to produce offspring. The number and geographic origin of the founder population was however not known. The current population size was 10 or more individuals, of which seven giraffe were sampled (but not all sequenced successfully). Overall, population PWR 4 had the highest diversity of the small Free State populations, followed by PWR 3, PWR 9, and lastly, PWR 6.

Due to the effect of small size, both census size and sample size, the data for the Free State populations were grouped into two metapopulations for further analysis - one containing all the small private Free State populations which excluded the municipal and Provincial Nature Reserves, and the largest private herd; and the alternative metapopulation consisting of all of the Free State populations pooled together. The rationale for this approach was that the many small giraffe populations in Central South Africa cannot reasonably be expected to add significant numbers of additional giraffe, due to the size of the areas and habitat availability. However, an approach where the small populations are managed as a bigger metapopulation, with occasional gene-flow facilitated by translocations, can contribute significantly to improved genetic management.

The term metapopulation can be defined as a group of local populations that are connected by dispersing individuals (Allendorf and Luikart 2007). This concept is generally based on the fact that larger habitat patches will be able to support larger populations. Habitat patches that are close to other occupied habitat patches will be recolonised. The migration of an individual to another habitat patch, will contribute to a decrease in the extinction rate based on demographic and genetic reasons (Allendorf and Luikart 2007). The advantage of this type of approach (from a management perspective) is that by looking at the populations while grouped, one can provide estimates of the overall contribution of the different subpopulations to total diversity (Allendorf *et al*. 2008). In the current giraffe study, the grouped populations had higher diversity, despite the fact that the individual small populations had low diversity. Both grouped populations had better diversity than most single populations, however, the diversity was a little higher when all of the Free State populations were combined compared to the level when only the small private Free State populations were included, suggesting that large Provincial reserve populations should ideally be included in a metapopulation approach. The diversity in both metapopulations were however, closely comparable to one another.

There are few examples where ungulate metapopulations were studied in southern Africa. There are however, some examples of metapopulation approaches using other taxa, namely elephant (*Loxodonta Africana*), lion (*Panthera leo*), rhino species and wild dogs (*Lycaon pictus*) (Hrabar and du Toit 2005; Allendorf *et al*. 2008,

Trinkel *et al*. 2010). In an article by Hrabar and du Toit (2005), it was stated that metapopulation management had been recommended for the conservation of black rhino, where the translocation of individuals among subpopulations was carefully considered, as to prevent adverse genetic and demographic conditions. By applying such a metapopulation approach, the successful reintroduction and management of wild dogs in South Africa was also possible (Trinkel *et al*. 2010).

In implementing a metapopulation approach for giraffe in Central South Africa, there should be well-planned exchanges of individuals from one subpopulation to another. It is recommended that the Free State as a metapopulation should be managed using a studbook, whereby genetic data and historical events pertaining to individual giraffe are indicated. This would ensure that individuals with the biggest potential for benefit are exchanged, rather than wasting resources on moving closely related animals. A metapopulation management plan should also consider aspects such as availability of animals, incidental opportunities for augmenting genetic diversity when animals become available on the market, and have due regard for veterinary implications. Finally, a comprehensive management plan could also include occasional inclusion of animals from further afield, notably the Limpopo and KwaZulu-Natal Provinces. The latter, however, requires careful consideration of genetic sub-structure and suitable units for conservation. This requirement, as well as detailed management recommendations, will be re-visited in the sections that follow.

4.2 Genetic differentiation and taxonomic status of giraffe populations from the Free State Province

Individuals sampled in the Free State and Northern Cape localities collectively exhibited two haplotypes for the Cyt *b* gene region, and 10 haplotypes for the D-loop region. It was inferred that there was 1 mutational event between the two Cyt *b* haplotypes, whereas there were between 1-9 mutational events between the 10 D-loop haplotypes. The pairwise differentiation between the localities in the Free State indicates little genetic variation based on the Cyt *b* gene, but more genetic variation based on the D-loop region.

Results from the current study provided valuable new data on the status of the Central South African giraffe population relative to the overall southern African giraffe population. The phylogenetic trees for both gene regions (from the current results and sequences obtained from GenBank) portray similar results. There is a definite divergence between subspecies found north and south of the Equator in Africa. The topologies generated during the present study are similar to that of Hassanin *et al.* (2007) and Bock *et al.* (2014). Both studies also found there was a separation between the populations north and south of the Equator, and similarly to the present study, Bock *et al.* (2014) found that the posterior probabilities of the northern mtDNA clade were low for some of the nodes. Bock *et al.* (2014) also stated that the matrilineal lineages found in their study, were congruent to the subspecies found previously, and reflect the geographic structure seen among giraffe.

Hassanin *et al.* (2007) and Bock *et al.* (2014) found that additional to the southern and northern divergence, there was further nucleotide divergence in the southern mtDNA clade, whereby *G. c. angolensis* occurred in the area to the west, and the other southern populations occurred in the area to the east. Hassanin *et al.* (2007) stated that additional nucleotide divergence in the southern mtDNA clade could have been the result of previous ecological or topographical barriers which prevented gene flow over time, ultimately leading to reproductive isolation in these lineages. Brown *et al*. (2007) agreed with Hassanin *et al.* (2007) that ecological or topographical factors could have had an impact on the distribution of the various subspecies, however, they also state that climate could have also had an impact. This was later confirmed by Deacon and Parker (2016).

Lorenzen *et al*. (2012) agrees with a north/south split as it coincides with patterns observed for hartebeest species (*Alcelaphus buselaphus*), roan antelope (*Hippotragus equinus*) and giraffe, and suggested that the equatorial forest belt could have caused the separation. Lorenzen *et al*. (2008) state that many African savannah ungulates are split into morphologically distinct species, which reflect evolutionary units. It is furthermore stated that the level of genetic differentiation varies among species. In this regard, giraffe are suggested to be genetically differentiated to such a degree that they should be recognised as distinct species. Lorenzen *et al*. (2012) also agrees that there is an east and west clade within hartebeest, roan, and giraffe. It is

affirmed that in hartebeest, waterbuck (*Kobus ellipsiprymnus*) and giraffe, the suture zone causing the north and south diverging lineage, is located in Kenya and Tanzania (Lorenzen *et al.* 2012).

Previously, nine giraffe subspecies were recognised - Dagg (1971); Ansell (1972); Dagg and Foster (1982); Kingdon (1997); East (1999); Grubb (2005); Ciofolo and Pendu (2013); Deacon and Parker (2016). Of these, four subspecies are found below the Equator in Africa, namely *G. c. angolensis*, *G. c. giraffa*, *G. c. thornicrofti*, and *G. c. tippelskirchi*. The subspecies found above the Equator are *G. c. antiquorum*, *G. c. camelopardalis*, *G. c. peralta*, *G. c. reticulata*, *G. c. rothschildi*. The distinctive clustering patterns observed within the two branches of the subspecies, can be as a result of geographical barriers found in the region. Bock *et al*. (2014) describe how the Owambo-Kalahari-Zimbabwe epeirogenis axis forms a subtle, yet distinct geography boundary between *G. c. angolensis* and *G. c. giraffa* populations.

The individuals sampled for the present study were all clustered into the southern mtDNA clade. Based on the D-Loop derived results, a total 39 individuals classified as *G. c. giraffe.* These animals were from populations MNR 1, PNR 2, PWR 1, PWR 3, PWR 5, PWR 6, PWR 8, PWR 9, PWR 10, PWR 11, PWR 13, PWR 16 and PWR 17. This is in line with expectations that locally sampled individuals should have mtDNA lineages of the South African giraffe. Unexpectedly, 28 individuals from the present study classified as *G. c. angolensis.* These individuals originated from populations MNR 2, PWR 2, PWR 4, PWR 11, PWR 14, PWR 15, and PWR 17. Furthermore, all animals sampled from central South Africa also displayed Cyt *b* haplotypes also displayed in *G. c. angolensis*. The trends from phylogenetic analysis were also apparent from F_{ST} and other measures of genetic differentiation. This outcome suggest that individuals with mtDNA lineages of the Angolan giraffe (*G. c. angolensis*) and not the South African giraffe (*G. c. giraffa*) are present in the Central South African giraffe population. These individuals may also be hybrids, either first generation or later, from crosses involving *G. c. giraffa* males and *G. c. angolensis* females.

The giraffe is a popular game ranching species, and there has been substantial commercial movement of the animal around the continent (Deacon and Parker 2016). The new findings, i.e. that some of the giraffe sampled in the Free State are Angolan

giraffe, exemplifies how the subspecies has been given a more southern distribution to what was previously thought, through translocations. This can be partly explained by the close historical link between South Africa and Namibia. Namibia was governed under control of the South African government until 21 March 1990, when Namibia gained its Independence (Cottrell 1991). The possibility of translocation of giraffe between Namibia and South Africa occurring in that time was good, as there was wide cooperation between the Namibian government and South African government. In fact, close collaboration between the two countries continues today. Individuals of *G. c. angolensis* could have found their way into South Africa along this route.

While the current results strongly suggest artificial mixing of subspecies, the relationship between translocation and the true nature of natural borders of subspecies should be carefully considered. Winter *et al*. (2018) discusses how the historically assumed distribution of the subspecies' ranged, and that the current distribution of *G. c. angolensis* ranges over a wider span than what was previously thought. Winter *et al*. (2018) also illustrated that only South African giraffe are assumed to be found in South Africa. Bock *et al.* (2014) however, found that from their study, two giraffe that had been assumed as South African giraffe, grouped with Angolan giraffe and not with the group it was expected to group with. These authors state that the assignment of giraffe to the incorrect subspecies was not unusual, and that it could be due to either natural migration or human-induced translocation. In their study, Bock *et al.* (2014) found that the South African giraffe was distributed more north than the previous known distribution. Also, Winter *et al*. (2018) found that the Angolan giraffe had a more eastern distribution than the previous known distribution. There is thus a need for a finer grained study of natural populations, to determine the true borders of subspecies.

There has been no reliable historical evidence of *Giraffa camelopardalis giraffa* occurring naturally in the Free State province (Deacon and Parker 2016). These authors stated that the translocation of the species into protected areas and private land in the Free State Province has caused an increase in numbers in the Province, regardless of the natural habitat preferences of giraffe. These authors also affirmed that giraffe had naturally occurred westwards of the Free State in the Northern Cape Province. The fact that the present study has found Angolan giraffe in the Free State,

reinforces Deacon and Parker's (2016) statement that Angolan giraffe have been translocated to areas within South Africa.

4.3 Management guidelines

Based on the results from the current study, the following guidelines for the genetic management of giraffe should be considered:

- To conserve diversity within population fragments and prevent the onset of inbreeding, the size and origin of founder groups should receive special attention, followed by facilitation of long-term gene flow.
- Since it is unlikely that a significant number of populations will increase their giraffe populations to a larger size, the only alternative is to manage in a way that will act to break total isolation of populations, is by exchanging individuals between populations.
- When populations are founded, managers or owners should aim to start with a founder population which is as large as possible, and/or with as many unrelated lineages as possible. Trinkel *et al*. (2010) state that the genetic diversity within a population depends on the size and genetic diversity of the founder population, followed by the rate at which new genes are added into the population. The introduction of new lineages into a population was also reiterated by Davies-Mostert (2009), when the long-term conservation potential should be considered.
- A Provincial database on giraffe should be implemented by FS DESTEA, whereby the historical management of the individuals should be captured. This should be done in order to keep track of where the giraffe originate from, and all other details pertaining to the individual. Following the creation of a Free State Provincial database, a database for other Provinces should be implemented, followed by a possible National database in the same format.
- Regulations pertaining to the import and export of different subspecies should be clearly described, disseminated to stakeholders and applied.

- The management of giraffe in protected areas, as well as private areas, should be recorded so that there is adequate record of current numbers and distribution.
- With regards to reintroductions and translocation, the level of genetic differences between surviving populations should be considered before mixing animals from different populations. Mixing of populations can result in long-term genetic consequences, such as reduced fitness (outbreeding depression; as seen in the Alpine ibex that lives in the European Alps (Taylor 2012)) and there is a danger of the expression of deleterious recessive alleles. Therefore, a conservation policy regarding the mixing of populations should be considered. The mixing of giraffe subspecies should be minimised or completely avoided.
- The populations found in natural distribution areas in South Africa should be compared to extralimital or introduced populations found in other South African Provinces, so that the effects of the mixing of subspecies can be investigated.
- The reintroduction of a giraffe into a new habitat should be followed by careful monitoring as there may have been reasons for the extinction of the animals in that particular habitat. These reasons should first be fully considered when reintroducing an individual to an area. Only once the cause of extinction has been determined and rectified, and there is adequate space to support a population, can animals be considered for reintroduction.

4.4 A notable additional outcome of the study – successful genotyping from faecal samples.

The use of non-invasive sampling has become a regular technique for sampling since its initial use, and many studies on various species has now been performed (Faria *et al.* 2011). The current study used non-invasive sampling methods to minimize the effect on the individuals. Faecal samples were collected, and DNA was isolated from these samples. Blood and tissue samples used for this study were collected for another study, and only used to supplement the main sample-set. With a total of 129 faecal samples collected, 78 individual samples (60%) amplified for the Cyt *b* gene, whereas 63 individual samples (49%) amplified for the D-loop region. Faecal samples

thus contributed 87% towards the study's samples, where blood contributed 11%, and tissue contributed 2%. Several similar studies done on giraffe used more invasive sampling techniques, such as tissue/skin biopsies (Brown *et al*. 2007, Brenneman *et al.* 2009a, Bock *et al*. 2014, Winter *et al*. 2018). A study which is comparable to the current study, was done by Stanton *et al*. (2014), whereby 37 Okapi faecal samples and 32 skin samples were collected to determine genetic structure of Okapi. Stanton *et al*. (2014) states that all of the faecal samples generated sequences. Faecal samples thus made up 54% of the study by Stanton *et al*. (2014).

The DNA quantities obtained from the faecal samples were lower than that obtained from tissue samples, but higher than the yield from blood samples. The DNA qualities from blood samples were lower than for tissue samples, but higher than quality from faecal samples. Piggott and Taylor (2003) stated that one of the drawbacks of using faeces is the low quality and quantity of DNA, which often lead to high rates of genotyping error. However, it was possible to generate 89 good-quality individual sequences during the current study. Contrary to the results of the present study, blood samples, in general, is one of the most widely used sources of both high quality and high quantity DNA for molecular purposes (Richardson *et al.* 2006). Richardson *et al.* (2006) however, did also state that long-term storage, where blood samples were frozen, resulted in a loss in DNA yield. These authors found that the longer the blood samples were stored at 4˚C, the lower the DNA yield was. Huang *et al.* (2017) agrees with Richardson *et al.* (2006) in stating that DNA quality from blood samples does not decline over short periods (15 days), but after a few weeks, DNA quality will start to decrease. This could explain the trends from the current study, since the blood samples used were comparatively old.

By using non-invasive techniques to attain faecal DNA samples, no harm is caused to the individual being sampled and no stress is placed on the animal. This technique was also seen as particularly convenient during the current study since reserve and farm owners allowed the researcher to pick up faecal samples without restriction, whereas if more invasive techniques were used, the majority if not all of the private owners would not agree to the sampling taking place.

The use of faecal samples, however, does come with certain challenges. Faria *et al.* (2011) mentions that the possible errors that are associated to this technique

includes variable defecation rates, unknown decaying times, and difficulty in identifying pellets of sympatric species. Another disadvantage of this technique is the time it took to ensure that the resampling of an individual was not done. The difficulty in identifying pellets of sympatric species was shown in a study done by Faria *et al.* (2011), who used non-invasive molecular techniques to confirm the presence of mountain bongo populations in Kenya. These authors collected 201 dung samples and found that various species could be identified by using the samples, and that despite high variation in the mtDNA control region in most antelope species, low genetic variation was found in mountain bongo. All of the dung samples from the study by Faria *et al.* (2011) successfully amplified and generated sequences.

For the present study, observation preceded collection of samples, thereby ensuring that target species were sampled and that it was fresh. By collecting fresh samples, DNA was relatively little degraded, as also reported by Beja-Pereira *et al*. (2009). The storage of samples in 96% ethanol (ETOH) followed, before samples were placed into a freezer. Beja-Pereira *et al*. (2009) state that ETOH has advantages such as preventing the formation of faecal powders, as well as keeping the external mucous layer (which contains sloughed cells cells) intact. A disadvantage of ETOH is that it is flammable, which complicates the transportation of samples, especially when 3rd parties assist by sending samples to a laboratory.

4.5 Recommendations for further study

To supplement and further explore the findings of the current study, the following recommendations are made for future study:

- Nuclear DNA markers can be added to the marker set used, to provide more detail on both diversity and differentiation. Similarly, additional mtDNA markers can provide valuable additional information.
- The inclusion of microsatellite markers, and ultimately SNP markers, could contribute to better fine-grained data on the population structure of the species, and can also be used to determine parentage and create pedigrees for individual populations.

- More sampling sites within broader South Africa would benefit a future study by expanding the database on genetic variants and remnants of diversity present in South Africa. Sampling outside South Africa should also continue, to provide data on the fine-grained distribution of genetic variants and subspecies, and indeed confirm the accuracy of previously published descriptions of subspecies boundaries and haplotype diversity.

In conclusion, the results of the present study contribute significantly towards the future conservation and management of giraffe in Central South Africa, by generating previously un-available data on the genetic characteristics of giraffe in the Free State Province. During the present study, the following two hypotheses were tested:

Hypothesis 1: Genetic diversity in giraffe populations is correlated to the degree of isolation.

Hypothesis 2: The extralimital giraffe population of Central South Africa was founded from one subspecies.

In concluding this M.Sc. project, it can be stated that from the results attained, Hypothesis 1 can be accepted, whereas Hypothesis 2 was found to be untrue.

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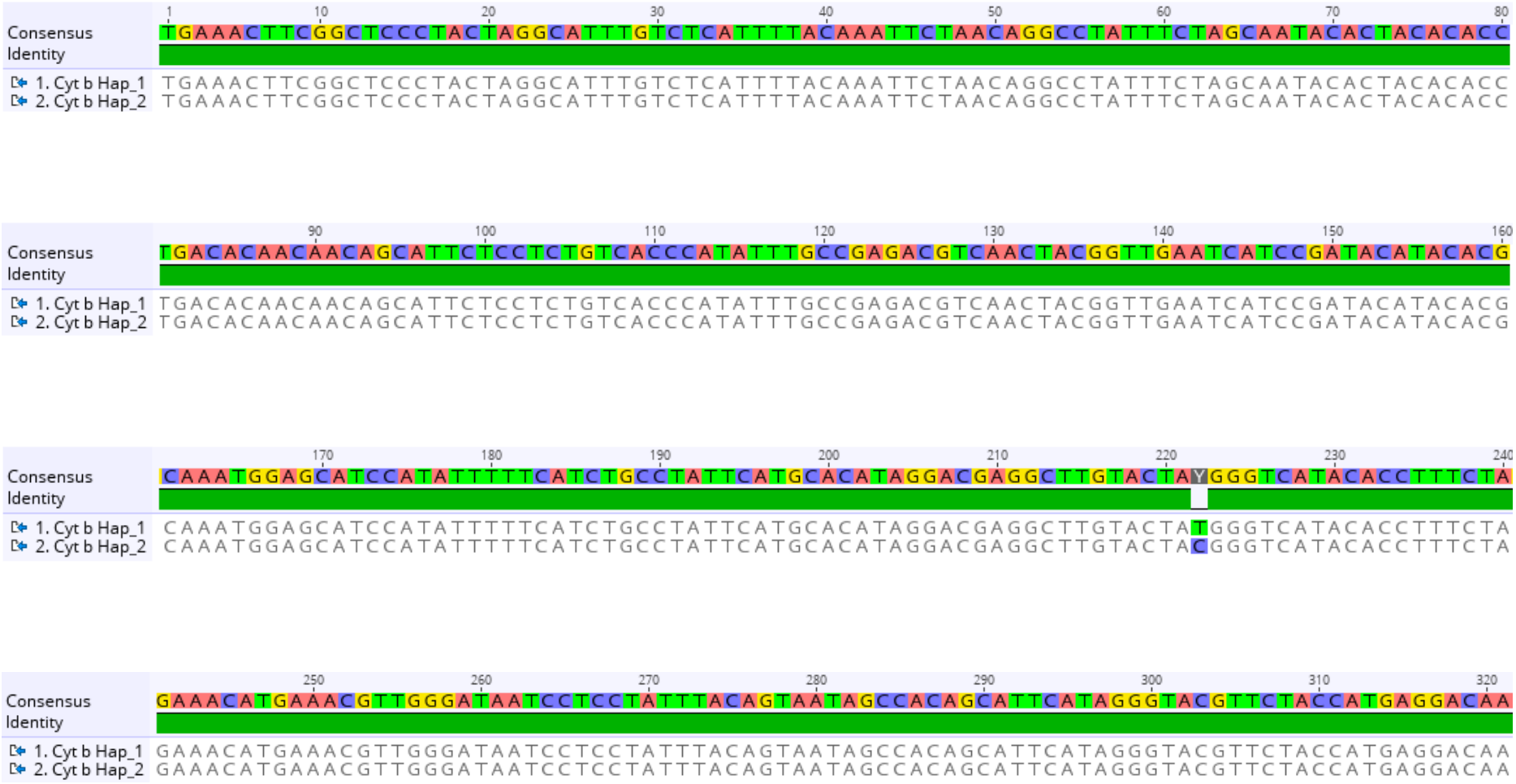
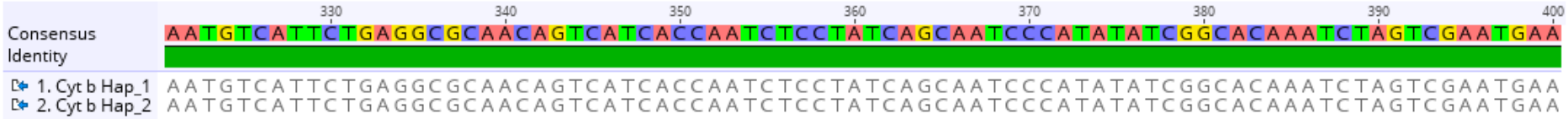
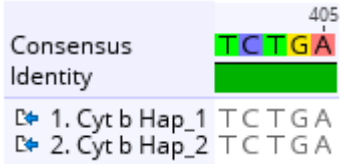
APPENDIX A:

Haplotype names and alignment sequences

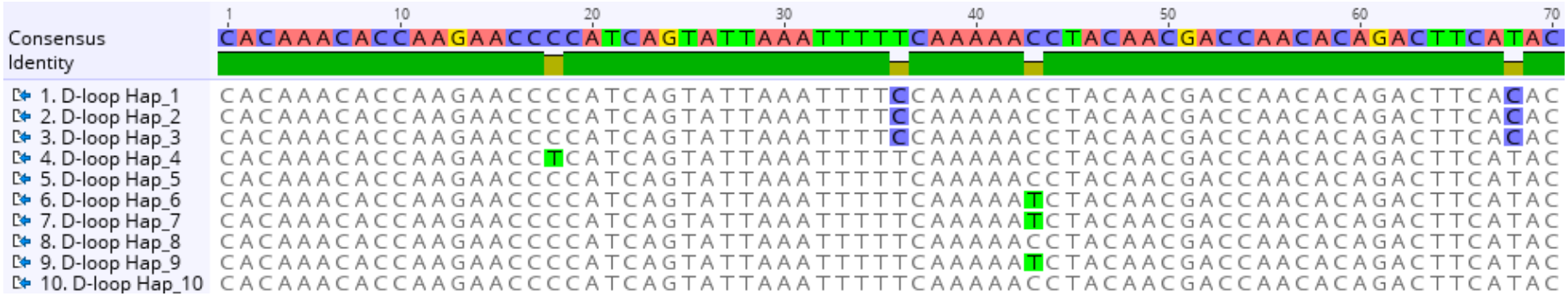
Cyt *b* Haplotypes

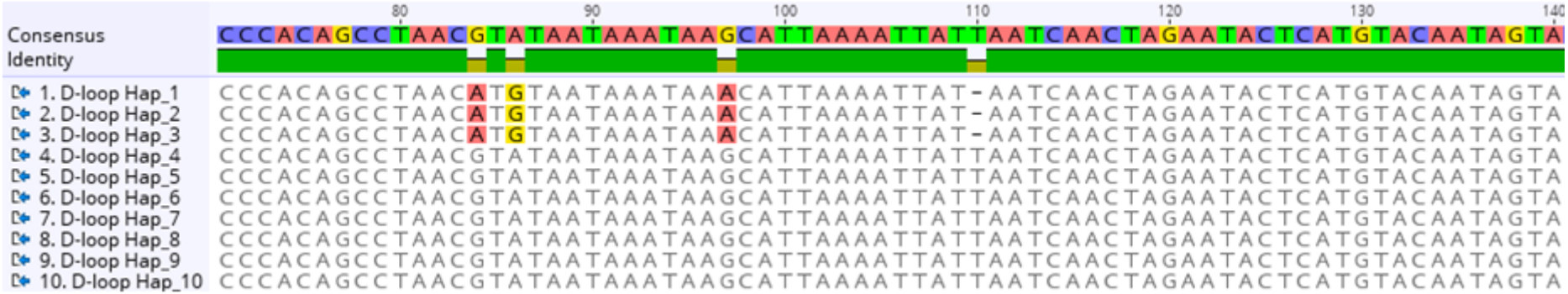


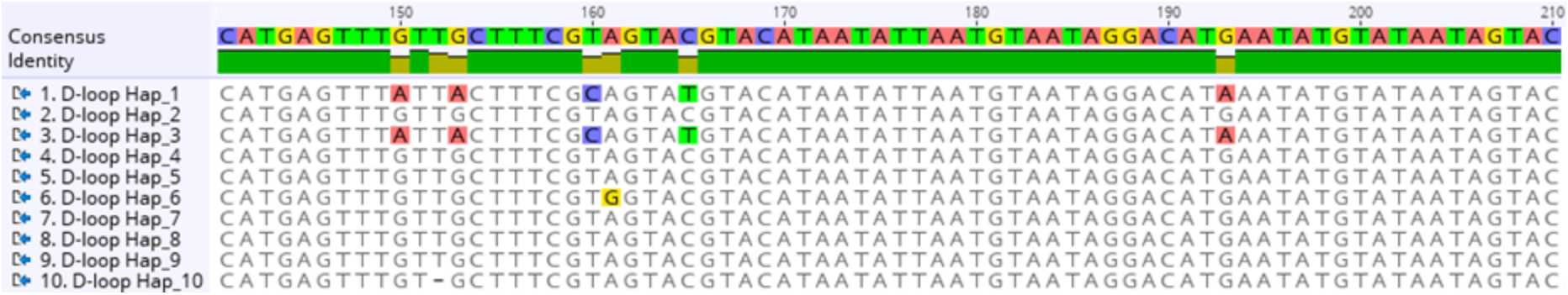

330
340
350
360
370
380
390
400
Consensus
Identity
1. Cyt b Hap_1 AATGTCATTCTGAGGCGCAACAGTCATCACCAATCTCCTATCAGCAATCCCATATATCGGCACAAATCTAGTCGAATGAA
2. Cyt b Hap_2 AATGTCATTCTGAGGCGCAACAGTCATCACCAATCTCCTATCAGCAATCCCATATATCGGCACAAATCTAGTCGAATGAA

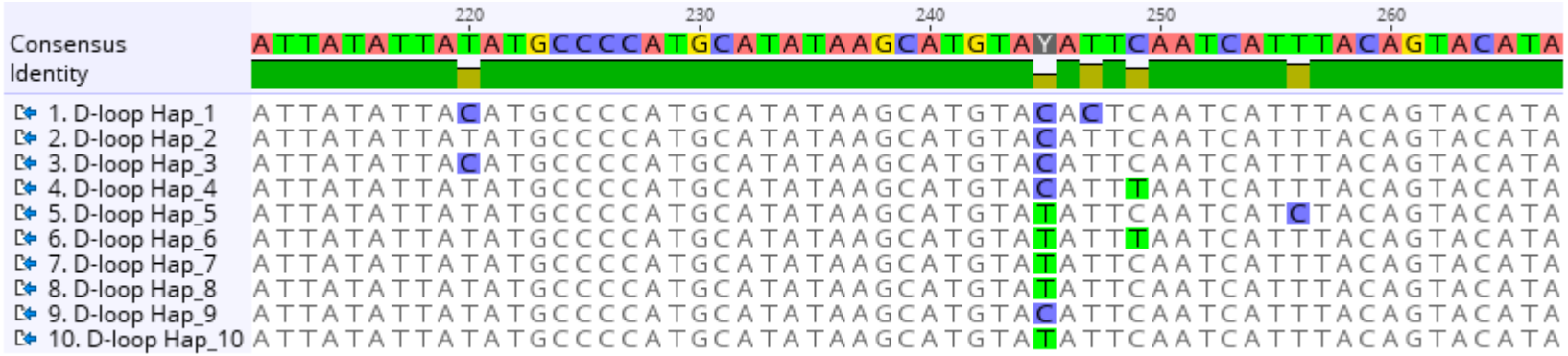

405
Consensus
Identity
1. Cyt b Hap_1 TCTGA
2. Cyt b Hap_2 TCTGA

D-loop Haplotypes






150
160
170
180
190
200
210
Consensus
Identity
1. D-loop Hap_1 CATGAGTTTATTACTTTCGCAGTATGTACATAATATTAATGTAATAGGACATAAATATGTATAATAGTAC
2. D-loop Hap_2 CATGAGTTTGTTGCTTTCGTAGTACGTACATAATATTAATGTAATAGGACATGAATATGTATAATAGTAC
3. D-loop Hap_3 CATGAGTTTATTACTTTCGCAGTATGTACATAATATTAATGTAATAGGACATAAATATGTATAATAGTAC
4. D-loop Hap_4 CATGAGTTTGTTGCTTTCGTAGTACGTACATAATATTAATGTAATAGGACATGAATATGTATAATAGTAC
5. D-loop Hap_5 CATGAGTTTGTTGCTTTCGTAGTACGTACATAATATTAATGTAATAGGACATGAATATGTATAATAGTAC
6. D-loop Hap_6 CATGAGTTTGTTGCTTTCGTGGTACGTACATAATATTAATGTAATAGGACATGAATATGTATAATAGTAC
7. D-loop Hap_7 CATGAGTTTGTTGCTTTCGTAGTACGTACATAATATTAATGTAATAGGACATGAATATGTATAATAGTAC
8. D-loop Hap_8 CATGAGTTTGTTGCTTTCGTAGTACGTACATAATATTAATGTAATAGGACATGAATATGTATAATAGTAC
9. D-loop Hap_9 CATGAGTTTGTTGCTTTCGTAGTACGTACATAATATTAATGTAATAGGACATGAATATGTATAATAGTAC
10. D-loop Hap_10 CATGAGTTTGT-GCTTTCGTAGTACGTACATAATATTAATGTAATAGGACATGAATATGTATAATAGTAC


220
230
240
250
260
Consensus
Identity
1. D-loop Hap_1 ATTATATTACATGCCCCATGCATATAAGCATGTACACTCAATCATTTACAGTACATA
2. D-loop Hap_2 ATTATATTATATGCCCCATGCATATAAGCATGTACATTCAATCATTTACAGTACATA
3. D-loop Hap_3 ATTATATTACATGCCCCATGCATATAAGCATGTACATTCAATCATTTACAGTACATA
4. D-loop Hap_4 ATTATATTATATGCCCCATGCATATAAGCATGTACATTTAATCATTTACAGTACATA
5. D-loop Hap_5 ATTATATTATATGCCCCATGCATATAAGCATGTATATTCAATCATCTACAGTACATA
6. D-loop Hap_6 ATTATATTATATGCCCCATGCATATAAGCATGTATATTTAATCATTTACAGTACATA
7. D-loop Hap_7 ATTATATTATATGCCCCATGCATATAAGCATGTATATTCAATCATTTACAGTACATA
8. D-loop Hap_8 ATTATATTATATGCCCCATGCATATAAGCATGTATATTCAATCATTTACAGTACATA
9. D-loop Hap_9 ATTATATTATATGCCCCATGCATATAAGCATGTACATTCAATCATTTACAGTACATA
10. D-loop Hap_10 ATTATATTATATGCCCCATGCATATAAGCATGTATATTCAATCATTTACAGTACATA