CZECH UNIVERSITY OF LIFE SCIENCES PRAGUE

Faculty of Tropical AgriSciences

Evaluation of Impact of Population Management on Genetic Parameters of Selected Spiral-horned Antelopes

MASTER'S THESIS

Prague 2023

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Declaration

I hereby declare that I have done this thesis entitled “Evaluation of Impact of Population Management on Genetic Parameters of Selected Spiral-horned Antelopes” independently, all texts in this thesis are original, and all the sources have been quoted and acknowledged by means of complete references and according to Citation rules of the FTA.

In Prague April 22, 2023

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Ema Cetkovská

Acknowledgements

My heartfelt appreciation goes to Mgr. Barbora Černá Bolfíková, Ph.D., who has been the best supervisor a student could ask for. She has guided me through the entire course of my studies, always ready to provide me with advice and encouragement. She has believed in my potential since the very beginning, and she did not give up on me on the days when I had given up on myself. I am also grateful to doc. Ing. Karolína Brandlová, Ph.D. for all the support and comments that were indispensable for the completion of this thesis, as well as for the many wonderful opportunities she has offered me.

I would like to thank Dr. Rob Ogden, who stands behind the idea for this thesis, for all his contributions and the materials he has provided me with. My appreciation equally goes to Ing. Kateřina Štochlová for all the time and effort she has put into this research and for all the knowledge she has shared with me. I hope that this thesis does justice to her work.

This thesis could not have been completed without the cooperation of EAZA and Nick Davis, Sander Hofman, Jörg Jebram, Beatrice Steck, Merel Zimmermann, and Peter Zwanzger, who have given me with access to studbook data and many other important pieces of information. Furthermore, I would like to extend my gratitude to everyone else who has, directly or indirectly, contributed to the completion of this thesis by involvement in sample collection and provision of materials or information.

I am equally indebted to the entire community of the Laboratory of Molecular Genetics for they have always been there for me to give me a helping hand.

I am eternally thankful to my parents for their unconditional love and continuous support. I know they have given up decades of their lives for me, I see all the sacrifices they make, and I do not take a single one for granted. My deep appreciation also goes to Míra for he has brought so much light and laughter into my life. Sometimes it only takes one star to brighten the darkest of skies.

This research was supported by the Internal Grant Agency of Faculty of Tropical AgriSciences (IGA 20223108; CIGA 20175006).

Abstract

Conservation of endangered animal species is one of the key objectives of captive populations, and its significance is expected to increase due to the rapidly growing number of vulnerable taxa. However, since these populations tend to be restricted in size and established using a limited number of founders, they commonly experience reduced genetic diversity, which might decrease their viability and ability to survive reintroduction into the wild. Although management has shown to have a positive effect on genetic parameters of such populations, it is frequently based on pedigree data, which may not be reliable, and inclusion of molecular methods is thus recommended. In this thesis, genetic characteristics of European Association of Zoos and Aquaria populations of six spiral-horned antelope taxa (mountain bongo, nyala, sitatunga, lesser kudu, greater kudu, common eland) were studied using a combination of microsatellite and mitochondrial markers and related to the current and past management of the populations, conservation statuses of the taxa, and availability of information useable for decision-making. The mitochondrial control region was also analyzed to identify geographic origin of the maternal lineages present in the captive populations and to evaluate the extent to which the captive stocks reflect the wild populations in terms of geographic and genetic representativeness. Several factors, including size and distribution range of wild populations, size of captive populations, number of founders, and thoroughness of management, were suggested as primary drivers affecting the values of the genetic parameters. High genetic diversity observed in sitatunga, greater kudu, and common eland may also be attributed to interbreeding of genetically distinct lineages, which raises a concern regarding the disruption of local adaptations. Consequently, genetic management should consider preventing interbreeding between the different clades. Furthermore, high inbreeding levels were found in mountain bongo and sitatunga, which should also be addressed in the future management of these populations. The origin of maternal lineages was successfully determined in all of the studied taxa, apart from the lesser kudu and mountain bongo, although in some instances solely as a broader region. Most of the studied captive populations were found to only mirror a portion of the wild distribution areas and to display a limited genetic representativeness.

Key words: captive populations, zoo management, Tragelaphini, mountain bongo, nyala, sitatunga, lesser kudu, greater kudu, common eland

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1. Introduction

Keeping of animals under human care serves a wide array of purposes, such as preservation of sufficiently large and viable animal collections representative of the corresponding wild populations (Lees & Wilcken 2009; Ballou et al. 2010), *ex-situ* conservation of endangered animal species and prevention of their extinction (Frankham 2008; Ballou et al. 2010; Witzenberger & Hochkirch 2011; Ralls & Ballou 2013; Wildt et al. 2019), subsequent reintroduction of individuals of these species into the wild (Lees & Wilcken 2009; Ballou et al. 2010; Witzenberger & Hochkirch 2011), support of *in-situ* conservation initiatives (Tribe & Booth 2003), education, and research (Tribe & Booth 2003; Lees & Wilcken 2009; Ballou et al. 2010; Witzenberger & Hochkirch 2011; Ralls & Ballou 2013; Wildt et al. 2019). The conservation aspect of captive populations is destined to increase in importance as the number of threatened species is growing at a rapid pace (Woodworth et al. 2002; Frankham 2008; Ralls & Ballou 2013).

Many captive populations are established using a small number of individuals (Leberg & Firmin 2008; Witzenberger & Hochkirch 2011), and their size is frequently further constrained due to restricted capacity of the facilities. This is especially problematic in the case of endangered species, whose wild populations already comprise a limited number of potential founders for captive populations, and the genetic variability of the captive stock is therefore severely diminished (Willoughby et al. 2015). Reduction of genetic diversity in captivity is attributed to several processes, such as genetic drift (Frankham 2008; Willoughby et al. 2015), inbreeding (Woodworth et al. 2002; Frankham 2008; Willoughby et al. 2015), and manifestation of deleterious alleles (Woodworth et al. 2002; Frankham 2008), with the impact of these mechanisms being more significant in small populations (Woodworth et al. 2002; Frankham 2008; Willoughby et al. 2015).

Thorough management of the captive populations was found to be effective in reducing the abovementioned negative impacts of small population size and retaining relatively favorable levels of demographic and genetic parameters, as well as the long-term viability of the populations (Ralls & Ballou 2013; Willoughby et al. 2017; Che-Castaldo et al. 2021; Putnam et al. 2023). However, most management strategies are based on pedigree

data issued from studbooks, the reliability of which may be hindered in case of their incompleteness or inaccuracy (Ivy & Lacy 2010; Norman et al. 2019). Moreover, several studies have shown that genetic parameters calculated from pedigree data may not veraciously reflect the actual situation within the captive populations (Willoughby et al. 2015; Ito et al. 2017), which is linked to the fact that analyses based on pedigree data assume unrelatedness of founders of the populations, and therefore tend to provide an overly positive view on the values of genetic diversity (Zemanová et al. 2015; Hogg et al. 2019). Consequently, inclusion of molecular data is advised to improve genetic management of captive populations (Ivy & Lacy 2010; Ivy et al. 2016; Norman et al. 2019; Jensen et al. 2020). Cooperative management, taking into account individuals from multiple institutions, rather than considering each zoo separately, is recommended (Che-Castaldo et al. 2021; Putnam et al. 2023). Moreover, genetic assessment of wild populations should also be performed to enable analysis of the genetic parameters (Ogden et al. 2018) and evaluation of the captive breeding programs in a wider context (Witzenberger & Hochkirch 2011).

The impact of management on genetic parameters has been studied in multiple animal taxa, including several antelope species. Intensively managed zoo populations of sable antelope (*Hippotragus niger*) were found to display higher levels of heterozygosity, reduced degree of inbreeding, and lower proportion of recent inbreeding events in comparison with the less managed ranch herds (Gooley et al. 2020). Similarly, highly controlled collections of scimitar-horned oryx (*Oryx dammah*) in zoos had higher heterozygosity and allelic richness than herds from ranches with minimal management. In addition to that, the Australian zoo population was characterized by high genetic diversity despite its small size, which was presumably a result of careful management (Ogden et al. 2020). A study comparing genetic parameters of an unmanaged captive stock of Cuvier's gazelle (*Gazella cuvieri*) from Morocco and of a Tunisian reintroduced population with a high degree of management revealed greater heterozygosity levels and lower inbreeding in the latter (Alvarez-Estape et al. 2022). On the contrary, Dicks et al. (2023) found similar values of genetic parameters in the well-organized zoo collections of addax (*Addax nasomaculatus*) and in a less managed population, nevertheless, this result may have been associated with a recent common ancestry of the herds. Although a group of animals from a different facility with a low level of management showed high

values of allelic richness, this situation was probably linked to a recent introduction of new individuals, and the population displayed greater inbreeding than the zoo collections (Dicks et al. 2023). The effect of management on genetic parameters was also examined by Kubátová et al. (2020) who came to the conclusion that the thorough organization of the semi-captive Western Derby eland (*Taurotragus derbianus derbianus*) herds in Senegal managed to maintain a low degree of inbreeding despite the small population size and limited number of founders.

This thesis is focused on examination of genetic parameters of European Association of Zoos and Aquaria (EAZA) captive populations, specifically the individuals found within EAZA institutions in European countries, with respect to their management, conservation statuses, and information availability, using six spiral-horned antelope taxa (mountain bongo, nyala, sitatunga, lesser kudu, greater kudu, and common eland) as a model. These taxa were selected due to their wide array of conservation statuses and management strategies, ranging from the common eland, a species given by the International Union for Conservation of Nature (IUCN) the status of least concern (IUCN SSC Antelope Specialist Group 2016b) and having no available studbook, to the critically endangered (IUCN SSC Antelope Specialist Group 2017) mountain bongo with an annually published studbook. Management intensity of the captive populations is evaluated based on data from the Zoological Information Management System (ZIMS) Species 360 database (2022) and studbooks, when available. Molecular analyses are performed using the biparentally inherited microsatellite markers to calculate genetic parameters of the populations and assess their structuring and the maternally inherited mitochondrial deoxyribonucleic acid (DNA) to reveal origin of the individuals, presence of genetically distinct clades, and the extent to which the captive populations represent the wild distribution area of the species and subspecies.

2. Aims of the Thesis

The first aim of the thesis is to assess genetic variability in European EAZA populations of six selected spiral-horned antelope taxa (mountain bongo, nyala, sitatunga, lesser kudu, greater kudu, common eland) and relate it to their past and present *ex-situ* management, conservation status, and availability of information useable in decision-making about further management of the populations. The second aim is to identify geographic origin of the different maternal lineages present in the European EAZA populations, compare it with the wild distribution area of the species and subspecies, and assess the genetic representativeness of the captive populations.

3. Literature Review

3.1. Biology, Conservation, and Genetics of the Selected Taxa

Spiral-horned antelopes, also known as Tragelaphini, are a clade of medium to large ungulate species named for their spiral-shaped horns (Frost 2014; Kingdon 2015; Castelló 2016). They belong to the Cetartiodactyla order, Bovidae family, and Bovinae subfamily (Groves & Grubbs 2011; Frost 2014; Kingdon 2015; Castelló 2016). Similarly to other antelope tribes, the taxonomy of Tragelaphini has undergone numerous changes in the recent history, especially with the advent of molecular methods, and a consensus is yet to be reached (Frost 2014).

According to the traditionally used biological concept of taxonomy, spiral-horned antelopes are typically classified into two genera and nine species. The *Tragelaphus* genus includes bongo (*Tragelaphus eurycerus*), bushbuck (*Tragelaphus scriptus*), greater kudu (*Tragelaphus strepsiceros*), lesser kudu (*Tragelaphus imberbis*), mountain nyala (*Tragelaphus buxtoni*), nyala (*Tragelaphus angasii*), and sitatunga (*Tragelaphus spekii*). The remaining two species, common eland (*Taurotragus oryx*) and giant eland (*Taurotragus derbianus*), are members of the *Taurotragus* genus (Kingdon 2015). Nevertheless, some sources recognize *Tragelaphus* as the sole genus of spiral-horned antelopes, comprising all nine species, and consequently use *Tragelaphus oryx* and *Tragelaphus derbianus* as scientific names for common and giant eland (East 1999; Frost 2014).

The more recent phylogenetic concept of taxonomy applied by Grubbs and Groves (2011) proposes the existence of five genera of spiral-horned antelopes: *Ammelaphus* for lesser kudu, *Nyala* for nyala, *Strepsiceros* for greater kudu, *Taurotragus* for common and giant eland, and *Tragelaphus* for bongo, bushbuck, mountain nyala, and sitatunga. In addition to that, the authors distinguish two separate species of lesser kudu, four species of greater kudu, and eight species of bushbuck. Sitatunga is provisionally classified into six species, nevertheless, one of them might require further division into multiple taxa (Grubbs & Groves 2011). Although some sources, such as Castelló (2016), started adopting the suggested phylogenetic concept, many authors and institutions, including the IUCN, still

adhere to the more traditional biological approach to spiral-horned antelope taxonomy (Frost 2014), and this system is thus applied in this thesis.

3.1.1. Mountain Bongo (*Tragelaphus eurycerus isaaci*)

Mountain bongo, also known as Kenya bongo or Eastern bongo (Castelló 2016), is one of the two recognized subspecies of *Tragelaphus eurycerus*. Despite being reported to have occurred in Uganda, mountain bongo became extinct there at the beginning of the twentieth century (East 1999), and it is nowadays endemic to Kenya (Frost 2014). The current distribution area is depicted in Figure 1. The subspecies inhabits montane forests of altitudes exceeding 2,000 meters above the sea level (Elkan & Smith 2013; Castelló 2016), favoring forest edges and disturbed forests with vegetation regrowth, therefore thriving on lands impacted by natural events, such as fires and landslides, as well as in areas moderately affected by logging (Elkan & Smith 2013; Frost 2014).

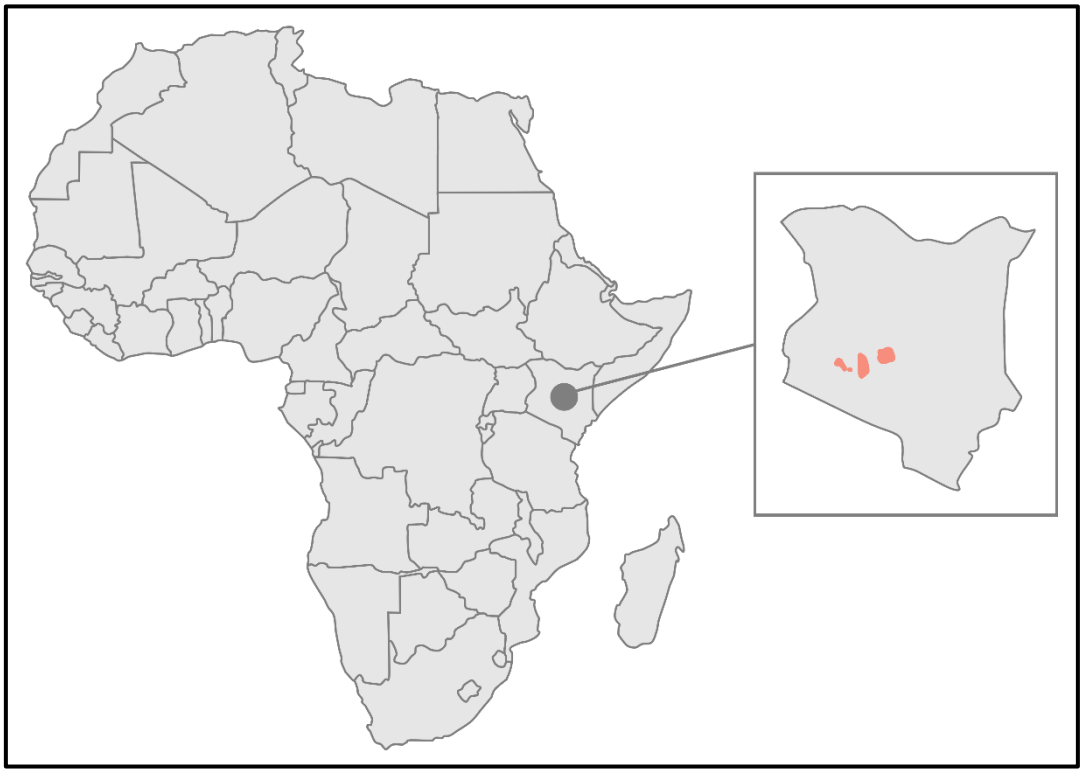

Figure 1: Mountain bongo distribution area; redrawn from IUCN SSC Antelope Specialist Group (2016a)

According to the latest IUCN assessment, the subspecies is considered to be critically endangered with a decreasing population trend (IUCN SSC Antelope Specialist Group 2017). No consensus on the precise number of individuals occurring in the wild has been reached, as Frost (2014) stated that there were fewer than two hundred animals, IUCN

SSC Antelope Specialist Group (2017) reported between seventy and eighty mature individuals, and Kenya Wildlife Service (KWS; 2019) estimated the total of 96 animals. The wild population is fragmented into five small subpopulations scattered throughout four distinct geographic areas: Aberdare Mountains, Mount Kenya, Eburu Forest, and Mau Forest (IUCN SSC Antelope Specialist Group 2017; KWS 2019), with the last one including two separate subpopulations. The Aberdare National Park is assumed to be inhabited by 40-50 individuals, the Mount Kenya National Park and Eburu Forest are expected to host six mountain bongos each, the Maasai Mau subpopulation is estimated to comprise 25 animals, and the southwestern portion of the Mau Forest Reserve presumably contains six to nine individuals. The situation seems to be the most alarming in the case of the Mount Kenya and Eburu groups since camera traps revealed minimal reproductive potential within these subpopulations, with no sighting of calves or males in the former and no record of females or young in the latter (KWS 2019). The study conducted by Sheppard et al. (2022) in the Eburu forest also detected no females of mountain bongo on the images from camera traps deployed within the area.

Subsistence and commercial hunting, habitat degradation and fragmentation due to grazing of domestic animals, and illegal logging are among the major threats impacting mountain bongo populations (Elkan & Smith 2013; IUCN SSC Antelope Specialist Group 2017; KWS 2019). In addition to that, the subspecies has been negatively affected by diseases, such as rinderpest, which contributed to the decrease in number of individuals at the end of the nineteenth and beginning of the twentieth century (IUCN SSC Antelope Specialist Group 2017), as well as during the 1980s (Mutu 2018). According to Elkan and Smith (2013), the negative effect of diseases on the mountain bongo population has commonly been underestimated, as illnesses were presumably among the major contributors to the population decline in the Aberdare Mountains. The number of individuals has equally been reduced by the presence of predators, including hyenas, leopards (KWS 2019; Sheppard et al. 2022), and lions (Elkan & Smith 2013; Frost 2014; Mutu 2018; KWS 2019).

In addition to the five wild subpopulations located in the montane regions of Kenya, a captive population was established within the Mt Kenya Wildlife Conservancy (MKWC) in 1967 (Mutu 2018), originating from individuals captured in the Aberdare

Mountains (Svengren et al. 2017). In 2004, the facility launched a breeding program (Mutu 2018) using 18 animals from the initial captive population, as well as 18 mountain bongos that were repatriated from American zoos (Bishop et al. 2019) with the intention to reinforce the wild population by individuals of captive origin. In March 2022, two females and three males were released to the newly established Mawingu Mountain Bongo Sanctuary, which aims to fully accustom the animals to wild conditions and produce offspring entirely independent of human intervention. From now onwards, ten bongos are expected to be moved from the conservancy to the sanctuary each year. In addition to that, the MKWC intends to improve genetic diversity of the population by introducing animals from European zoos (Mutu 2022).

Several studies concerning genetics of the subspecies have been published in the past two decades. Faria et al. (2011) identified the presence of two haplotypes, B01 and B02, differing from one another by a single nucleotide transition, within the wild population of mountain bongo. The B02 haplotype was found to be predominant, represented by approximately 70% of the samples, and it was the only haplotype detected in individuals from the Eburu and Mau Forests. Furthermore, the number of mountain bongo haplotypes was lower compared to that discovered in a sympatrically living population of waterbuck (*Kobus ellipsiprymnus*), implying low genetic variation found within the Kenyan wild population of mountain bongos (Faria et al. 2011).

A study published by Svengren et al. (2017) compared genetic parameters of Kenyan wild and captive mountain bongo populations using single nucleotide polymorphisms (SNPs), observing negative values of inbreeding coefficient and low degree of relatedness among individuals despite the small sizes of the populations. O'Donoghue et al. (2017) studied the captive bongos in European zoos by analyzing mitochondrial DNA, providing the first genetic evidence for the existence of the two bongo subspecies, mountain bongo (*Tragelaphus eurycerus isaaci*) and lowland bongo (*Tragelaphus eurycerus eurycerus*). Furthermore, the authors only identified the B01 haplotype, which was found to be underrepresented in the wild by Faria et al. (2011), in the European zoo population (O'Donoghue et al. 2017). Similarly, all EAZA and Kenyan captive mountain bongo samples analyzed by Sandri (2020), were attributed the B01 haplotype, despite the author erroneously labeling it as B02, which may be associated with the fact that the haplotype

names assigned to the sequences in the National Center for Biotechnology Information (NCBI) Nucleotide database do not follow the original naming introduced in Faria et al. (2011).

The diminished genetic diversity present within the wild mountain bongo population (Faria et al. 2011; O'Donoghue et al. 2017; Svengren et al. 2017) highlights the need for implementation of conservation measures to protect the subspecies from the impact of stochastic events. While translocations among the different Kenyan subpopulations represent one possible solution (Svengren et al. 2017), the discovery of different haplotypes in the wild and captive populations revealed the potential for the EAZA mountain bongo stock to serve as a suitable source of animals for reinforcement of the Kenyan wild population (O'Donoghue et al. 2017). Furthermore, O'Donoghue et al. (2017) equally highlighted the advantageousness of a two-way exchange of animals as addition of Kenyan mountain bongos to the European captive population could improve its long-term genetic viability.

3.1.2. Nyala (*Tragelaphus angasii*)

Nyala, also referred to as common or lowland nyala (Castelló 2016), is a spiral-horned antelope inhabiting southeastern Africa ranging from Malawi to South Africa (East 1999; Frost 2014; Castelló 2016; IUCN SSC Antelope Specialist Group 2016d). While it is native to Malawi, Mozambique, Zimbabwe, South Africa, and Eswatini, the presence of nyala in eastern Botswana and Namibia (East 1999; Castelló 2016; IUCN SSC Antelope Specialist Group 2016d) is a consequence of introduction to private lands, and the species does not occur in the wild within these countries (IUCN SSC Antelope Specialist Group 2016d). Nyala became extinct in Eswatini during the 1950s (Castelló 2016; IUCN SSC Antelope Specialist Group 2016d) but was later successfully reintroduced to natural reserves and private ranches (East 1999). The distribution range is presented in Figure 2. Habitats typically occupied by nyalas are characterized as thickets and woodlands in proximity of water sources (Frost 2014; IUCN SSC Antelope Specialist Group 2016d).

F**igure 2:** Nyala distribution area; redrawn from IUCN SSC Antelope Specialist Group (2016d)

The species is attributed the conservation status of least concern with a stable population trend (IUCN SSC Antelope Specialist Group 2016d). There are more than 30,000 animals present worldwide (Frost 2014; Castelló 2016), and approximately 20,000 to 27,500 of these individuals are mature (IUCN SSC Antelope Specialist Group 2016d). The largest portion of the animals is located in the KwaZulu-Natal province in South Africa (Frost 2014; Castelló 2016), which hosts around 25,000 nyalas (Castelló 2016). More than 80% of the populations are found on protected areas and 10-15% inhabit private land (Castelló 2016; IUCN SSC Antelope Specialist Group 2016d). Spreading of nyalas outside of areas of original distribution is aided by grazing of grasslands by domestic animals, which promotes bush encroachment and consequently renders the land more suitable for nyalas (Frost 2014). Furthermore, since the species is popular among trophy hunters (Anderson 2013; Frost 2014; Castelló 2016) and tourists, translocations of individuals among farms and regions are relatively common (Grobler et al. 2005). Agriculture expansion, hunting, and diseases represent the most important factors negatively impacting the species (IUCN SSC Antelope Specialist Group 2016d). Although the combination of hunting and rinderpest caused the extinction of nyala in Eswatini in the middle of the twentieth century (East 1999), there are nowadays no major threats affecting the species (IUCN SSC Antelope Specialist Group 2016d).

A study focused on genetics of nyala was conducted by Grobler et al. (2005) since the translocations, exchanges of individuals among different regions, and fragmentation of the population into small groups of reproductively isolated animals raised a concern regarding their potential negative impact on the genetic parameters of the species. The haplotype network constructed using mitochondrial DNA revealed the presence of two main clusters, one comprising samples from Malawi and Mozambique, and the other consisting of animals from South Africa and Zimbabwe, which were separated by the minimum of seven mutation steps. Due to the genetic distinctiveness of the two clades and consequent potential presence of local adaptations, the authors proposed to treat the groups as separate evolutionary significant units and discouraged translocations of animals between them, at least until the existence of local adaptations is further investigated (Grobler et al. 2005).

3.1.3. Sitatunga (*Tragelaphus spekii*)

Sitatunga is a spiral-horned antelope widespread throughout sub-Saharan Africa. It mostly inhabits the central portion of the continent and the Congo Basin, but the distribution range also extends eastwards to Kenya, Tanzania, Uganda, Rwanda, Burundi (Frost 2014), and potentially Ethiopia (Castelló 2016). The southernmost places of its occurrence are found in Botswana, the Caprivi Strip of Namibia (Frost 2014), and Zimbabwe (Castelló 2016). Populations of sitatungas are equally present in western Africa, with the westernmost point being Senegal and Gambia (Frost 2014). The distribution range is depicted in Figure 3. Sitatungas populate habitats with dense vegetation cover and proximity of water (Frost 2014; IUCN SSC Antelope Specialist Group 2016e), such as swamps, humid forests, and riverine thickets (Frost 2014; Kingdon 2015; Castelló 2016; IUCN SSC Antelope Specialist Group 2016e).

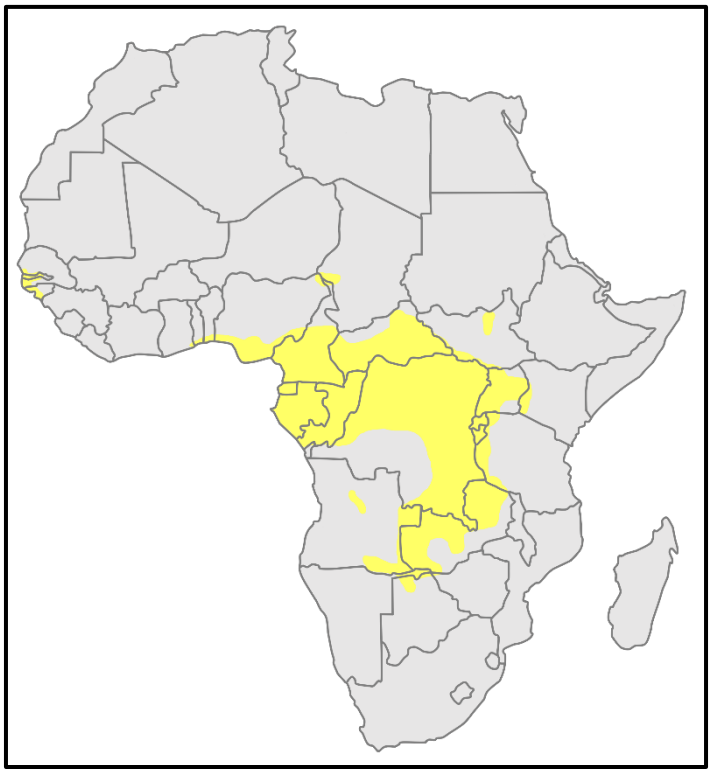

Figure 3: Sitatunga distribution area; redrawn from IUCN SSC Antelope Specialist Group (2016e)

Sitatunga is attributed the conservation status of least concern with a decreasing population trend (IUCN SSC Antelope Specialist Group 2016e). Estimation of population size is rendered difficult by high vegetation density within the habitats occupied by sitatungas (Starin 2000; Frost 2014), as well as the cryptic nature of the animals (IUCN SSC Antelope Specialist Group 2016e). Frost (2014) stated that the total population might attain 150,000 individuals, and IUCN SSC Antelope Specialist Group (2016e) provided an estimate of 90,000 to 120,000 mature animals. Forty percent of sitatungas occur within the ranges of protected areas (East 1999; IUCN SSC Antelope Specialist Group 2016e). According to Frost (2014), the population decline is occurring over the entire area of distribution, apart from core regions, whereas IUCN (IUCN SSC Antelope Specialist Group 2016e) claims that the decrease in number of individuals is only affecting densely habited locations. Nevertheless, the overall population decline does not exceed twenty percent over the course of three generations. While relatively rare in western Africa (IUCN SSC Antelope Specialist Group 2016e) and certain localities in Kenya, Chad, and Zimbabwe (Kingdon 2015), the species is still relatively common over the majority of its distribution range (IUCN SSC Antelope Specialist Group 2016e).

Loss of wetland habitats and changing water levels affecting vegetation composition represent the primary threat to sitatungas, as fragmentation of habitat results in the creation of small and isolated groups of animals (May & Lindholm 2013; IUCN SSC Antelope Specialist Group 2016e). The species is also negatively impacted by hunting for meat (Frost 2014; Kingdon 2015; IUCN SSC Antelope Specialist Group 2016e) and competition with livestock (Castelló 2016). In addition to that, Brichieri-Colombi et al. (2017) identified expansion of agricultural land and climate change as factors endangering sitatungas in Ghana.

No study focused on genetics of sitatunga has been published. Brichieri-Colombi et al. (2017) investigated the case of sitatunga in Ghana, and calculated the maximum effective population size to be 840 individuals based on habitat suitability model. Since the value is lower than would be desirable for long-term survival, the authors suggested that augmentation of gene flow would be beneficial. However, this conclusion is not backed up by any genetic data (Brichieri-Colombi et al. 2017).

3.1.4. Lesser Kudu (*Tragelaphus imberbis*)

Lesser kudu is a Tragelaphini antelope inhabiting the horn of Africa (Leuthold 2013), more specifically Ethiopia, Kenya, Tanzania, Somalia (East 1999; Frost 2014; Castelló 2016; IUCN SSC Antelope Specialist Group 2016c), South Sudan, and Uganda (East 1999; Castelló 2016; IUCN SSC Antelope Specialist Group 2016c). The species formerly occurred in Djibouti but presumably became extinct within this country (IUCN SSC Antelope Specialist Group 2016c). In addition to that, the population in Somalia underwent a significant reduction between the 1980s and 1990s and likely disappeared from the central and northern portion of the country. Even though the number of animals in southern Somalia was thought to be relatively high (East 1999), Frost (2014) claimed that the species might have become extinct from the entirety of the country. Lesser kudu has also been reported to inhabit Eritrea, however, its occurrence in the area has not been confirmed. Precise geographic limits of lesser kudu distribution are debatable, which may partially be attributed to the common confusion of the species with greater kudu due to their physical similarities and overlap of distribution range (Leuthold 2013). The current distribution of the species is depicted in Figure 4. Lesser kudu inhabits thickets and bushlands with an abundance of *Accacia* and *Commiphora* (East 1999; Kingdon 2015;

IUCN SSC Antelope Specialist Group 2016c), while avoiding open spaces (Frost 2014). The species is found in drier areas than greater kudu (Frost 2014; Kingdon 2015).

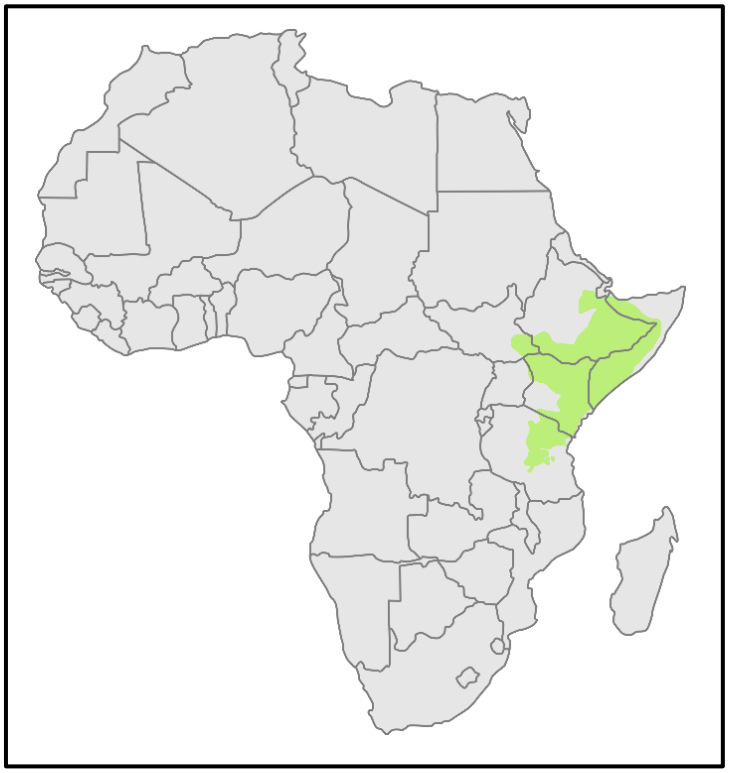

Figure 4: Lesser kudu distribution area; redrawn from IUCN SSC Antelope Specialist Group (2016b)

The species is assigned the status of near threatened with a decreasing population trend (IUCN SSC Antelope Specialist Group 2016c). East (1999) proposed the total number of 118,000 individuals based on aerial surveys and correction for bias associated with this method of population size assessment. Frost (2014) stated that the number of lesser kudu exceeds 100,000 animals, and IUCN SSC Antelope Specialist Group (2016c) provided an estimate of 80,000 to 100,000 mature individuals. The difficulty of population size estimation may be attributed to the cryptic behavior of the species and its preference for dense habitats (Leuthold 2013).

Only a third of the world population occurs in protected areas (East 1999; Frost 2014), and it is threatened by expansion of human settlement (Castelló 2016), excessive hunting, and competition with livestock (Frost 2014; Castelló 2016). Nevertheless, Kingdon (2015) stated that the species might benefit from overgrazing as it reduces the risk of fires that would make the habitats unsuitable for the animals. Lesser kudus are also endangered by the unstable political situation in the region as armed conflicts lead to higher

prevalence of weapons among people and consequent elevated hunting pressure, reduced control over protected areas, and difficulty to obtain accurate information on the population status (Leuthold 2013). The species is also susceptible to rinderpest, with the 1994-1995 outbreak in Kenya further reducing the number of animals, which had already suffered a 50% decline from the 1970s to the early 1990s. However, this disease only had minimal influence on the population size in Ethiopia (East 1999). Since the shy nature of the animals provides them with a certain degree of protection (Kingdon 2015), the species seems to be able to cope with high hunting pressure in dense habitats (East 1999; IUCN SSC Antelope Specialist Group 2016c), and it is not immediately threatened (Leuthold 2013). Nonetheless, the population decline is expected to exceed 25% within three generations (Steck 2022), and measures should thus be taken to prevent a significant population decline due to habitat fragmentation in the future (Leuthold 2013).

The sole large-scale genetic study of lesser kudu was conducted by Bock et al. (2014), who compared captive animals from American and European zoos, as well as a wildlife park in Dubai. The results showed a high mitochondrial diversity in comparison with nuclear diversity, which was attributed to the fact that the composition of founder population was skewed towards females. The animals from Dubai, which are unique due to their Somalian origin, each represented a different haplotype, unshared with any individuals from other institutions, nevertheless, they failed to create a separate group in the haplotype network. The authors equally concluded that use of microsatellites would be beneficial for further genetic assessment of the population (Bock et al. 2014).

3.1.5. Greater Kudu (*Tragelaphus strepsiceros*)

Greater kudu is a spiral-horned antelope widespread in sub-Saharan Africa, in a large belt expanding from Angola, Zambia, and Mozambique to Namibia, Botswana and northern South Africa, with a geographically isolated subpopulation in central South Africa. The distribution range also extends to eastern Africa, where greater kudus occupy areas in Eritrea, Ethiopia, Kenya, and Sudan (Frost 2014), with Castelló (2016) equally mentioning Somalia and Uganda among the list of countries with presence of greater kudus. However, Frost (2014) claimed the species to be extinct in Somalia, and East (1999) equally considered this extinction likely. The westernmost populations are observed in an area shared by Chad, Central African Republic, Sudan, and South Sudan

(Frost 2014; Kingdon 2015; Castelló 2016). The distribution range of greater kudu is presented in Figure 5. The species resides in various savanna habitats (Owen-Smith 2013; Frost 2014), ranging from dense woodlands (Kingdon 2015) to thickets (Kingdon 2015; Castelló 2016) and scrub woodlands (Castelló 2016; IUCN SSC Antelope Specialist Group 2020).

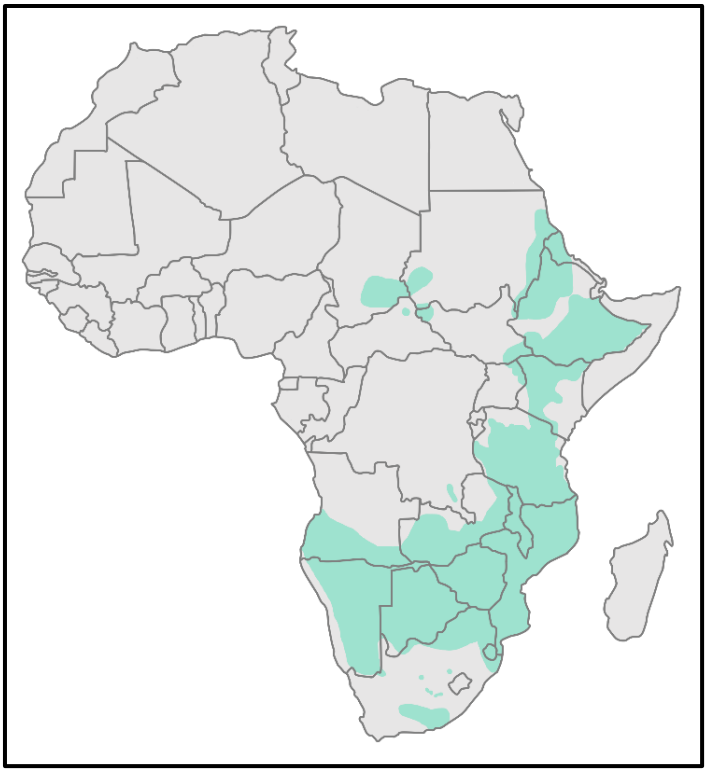

Figure 5: Greater kudu distribution area; redrawn from IUCN SSC Antelope Specialist Group (2020)

The species is assigned the status of least concern with a stable population trend (IUCN SSC Antelope Specialist Group 2020). The population is estimated to contain between 300,000 and 500,000 greater kudus, depending on the applied method of survey (East 1999; IUCN SSC Antelope Specialist Group 2020). According to East (1999), 61% of the population live on private land, and 15% inhabit protected areas. The number of animals is generally increasing in these regions (IUCN SSC Antelope Specialist Group 2020), with East (1999) specifically highlighting the population growth in southern and south-central Africa, as well as in Tanzania. On the contrary, the remaining 24% of the distribution range suffer from reduction in population size (IUCN SSC Antelope Specialist Group 2020). The situation is more critical in the northern portion of greater kudu presence (East 1999; Frost 2014; IUCN SSC Antelope Specialist Group 2020), where the subpopulations tend to be more fragmented and declining in numbers (East

1999). Frost (2014) also attributes the more favorable state in southern Africa to the high popularity of the species among trophy hunters who contribute to its conservation. In addition to that, the status of the greater kudus inhabiting central and northern Africa is worsened by the lack of demographic data (Frost 2014; Castelló 2016). The species has already become extinct in Djibouti (IUCN SSC Antelope Specialist Group 2020), which may have been associated with excessive hunting followed by habitat degradation (East 1999). According to Kingdon (2015), greater kudu is endangered in Uganda, vulnerable in Chad and Kenya, and its distribution range in Tanzania is constantly shrinking.

Although the species has disappeared from many core regions, it continues to occur over the majority of its original area of distribution (East 1999; IUCN SSC Antelope Specialist Group 2020), which may be associated with the ability of greater kudus to survive in proximity of human settlements and the secretive nature of the animals. Greater kudus are primarily threatened by habitat loss and hunting (IUCN SSC Antelope Specialist Group 2020) for both meat and trophies (Owen-Smith 2013). Nevertheless, the overall negative impact of hunting on the population is reduced by the ability of the animals to withstand the hunting pressure and their abundance in protected areas, and hunting is thus not expected to significantly affect the long-term viability of the species (IUCN SSC Antelope Specialist Group 2020).

A research on genetics of greater kudu was conducted by Nersting and Arctander (2001) using mitochondrial DNA. The phylogenetic tree revealed the presence of three clades, with the most basal one consisting of individuals from South Africa and Namibia. Distinction of the other two clades, one containing animals from eastern Africa, and the other comprising samples from southern Africa, the intermediate zone of Zambia, and several specimens from southwestern Africa, was only weakly supported. The authors associated the high degree of genetic differentiation of the southwestern population with the fact that the area served as a refugium for animals adapted to arid conditions during Pleistocene (Nersting & Arctander 2001).

Genetic research was also done by Sakwa (2001), who used both mitochondrial DNA and microsatellites to assess the greater kudu wild populations. The analysis of mitochondrial DNA distinguished two groups, one containing haplotypes from Namibia and South

Africa, while the samples from both of these countries, as well as Botswana, Zambia, Zimbabwe, and Chad, were present in the other clade. Similarly, presence of two groups was revealed by the phylogenetic tree constructed using microsatellite data: one with Tanzanian, Namibian, and Zambian animals, and the other containing samples from South African and Botswanan localities. The pairwise fixation index among the different sampling localities was higher for mitochondrial DNA compared to microsatellite markers, which was attributed to the fact that males display higher mobility than females, which causes their contribution to gene flow to be higher, and consequently a greater variability of biparentally inherited markers contrary to the maternally inherited ones. The lack of strong phylogeographic structure was also linked to climate changes during Pleistocene, with the diminished dispersal and geographic isolation of the different populations during glacial periods presumably leading to reduction of the overall genetic variability of the species due to bottlenecks and founder effects (Sakwa 2001).

The South African population of greater kudu was also studied by Jacobs et al. (2022), who found highest genetic diversity in the north with a gradual decline towards the southern portion of the country, which was attributed to the fact that the populations in the northern regions resisted the decline in number of animals at the beginning of the nineteenth century due to protection, which preserved their genetic diversity. The maximum-likelihood phylogenetic tree revealed two clades: western clade, containing mostly samples from the south and west of the country, and eastern clade, comprising individuals from the northern and eastern portion of South Africa. The western group clustered with the South African and Namibian clade identified by Nersting and Arctander (2001), whereas the eastern group adhered to the intermediate clade. The samples from the central region of South Africa were scattered throughout both of the groups. The haplotype network equally showed the presence of two groups with similar distribution of samples based on their geographic origin (Jacobs et al. 2022).

3.1.6. Common eland (*Taurotragus oryx*)

Common eland is found in eastern and southern Africa (Thouless 2013; Frost 2014; IUCN SSC Antelope Specialist Group 2016b) from South Sudan and Ethiopia to South Africa (Frost 2014; Castelló 2016). According to Castelló (2016), the species is also present in Lesotho and Eswatini, whereas Frost (2014) expanded the distribution area by addition

of Rwanda, Burundi, and Angola. However, certain sources stated that the common eland is extinct in Burundi (East 1999; Castelló 2016; IUCN SSC Antelope Specialist Group 2016b), and its presence in Angola is debatable (East 1999; Castelló 2016). The map of current distribution of the species is presented in Figure 6. The common eland inhabits a wide array of habitats, ranging from arid areas (Thouless 2013; Frost 2014) through Mediterranean vegetation (Frost 2014; Castelló 2016) to open woodlands, avoiding true deserts and dense forests. It occupies a range of altitudes from coastal regions to almost 5,000 meters above the sea level (Thouless 2013).

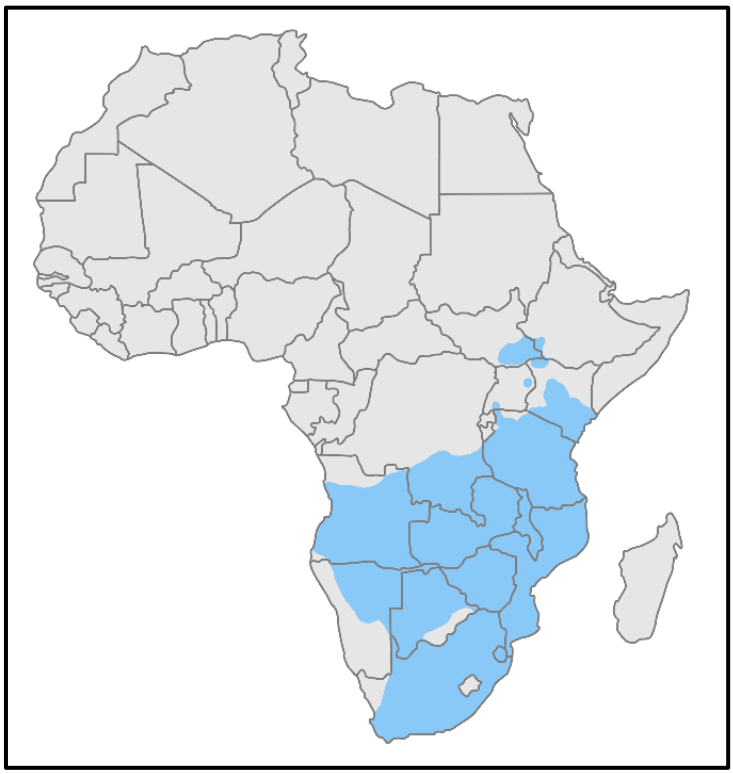

Figure 6: Common eland distribution area; redrawn from IUCN SSC Antelope Specialist Group (2016b)

The species is assigned the conservation status of least concern with a stable population trend (IUCN SSC Antelope Specialist Group 2016b). East (1999) estimated the global population to contain 136,000 individuals based on aerial counts and correction for bias caused by the survey method. Similar numbers were provided by other authors, with Frost (2014) stating that there were over 100,000 common elands in the world and IUCN SSC Antelope Specialist Group (2016b) estimating the total number of mature animals to be between 90,000 and 100,000. The largest populations are found in Namibia and Tanzania (Thouless 2013). According to East (1999), half of the population is located in protected areas and additional 30% on private land.

Common elands are threatened by hunting (East 1999; Thouless 2013; Frost 2014; Kingdon 2015; IUCN SSC Antelope Specialist Group 2016b) due to the high quality and quantity of their meat (Thouless 2013; Frost 2014). Moreover, the species is negatively affected by human settlements expansion and livestock production (East 1999; IUCN SSC Antelope Specialist Group 2016b). Fragmentation of habitat also negatively impacts common elands (Thouless 2013; IUCN SSC Antelope Specialist Group 2016b) as it prevents them from exhibiting their natural tendency to move over large areas and increases their susceptibility to drought and diseases (Thouless 2013), with these factors being responsible for a larger than tenfold population decline in Kenya over the course of the 1990s (East 1999). Furthermore, the species is threatened by political instability (IUCN SSC Antelope Specialist Group 2016b), which is apparent in the population size reduction in Mozambique in the 1980s and 1990s (East 1999). Generally, the number of common elands is growing on private land (Frost 2014; IUCN SSC Antelope Specialist Group 2016b), the population trend is variable in different protected areas (IUCN SSC Antelope Specialist Group 2016b), and a decline is observed in all other regions (Frost 2014; IUCN SSC Antelope Specialist Group 2016b). The species is not immediately threatened provided that the populations in protected areas and private ranches are maintained at current levels (Thouless 2013), the feasibility of which Frost (2014) believes to be aided by the fact that common elands provide significant economic benefits to the landowners. In addition to that, the situation has profited from introduction of common elands to many ranches, particularly in southern Africa (Thouless 2013).

A study focusing on genetics of common elands was published by Lorenzen et al. (2010), with the phylogenetic tree revealing three lineages of common elands, labeled southern, eastern, and intermediate, based on their geographic distribution. The southern lineage contained individuals from southern Africa, as well as several samples from Tanzania, the eastern lineage was comprised of eastern African animals, and the intermediate lineage united three haplotypes from Zimbabwe. Geographic structuring was more prominent in the eastern clade, with two groups containing individuals from southwestern Tanzania, while the other two groups were composed of animals from northeastern Kenya, Uganda, and Ethiopia, as well as two haplotypes from northern Tanzania. Particularly high nucleotide diversity was observed in one locality in Tanzania and one

in Zimbabwe due to the mixing of intermediate and eastern clades, and intermediate and southern clades, respectively (Lorenzen et al. 2010).

The southern lineage of common elands is presumably older than the eastern one, based on the high genetic diversity, as well as the lower haplotype structuring and genetic differentiation values among the different populations, implying the presence of long-lasting gene flow. However, the authors concluded that the difference in genetic parameters between the two lineages may have been impacted by a recent bottleneck event in eastern Africa, as well as the smaller population size of the eastern lineage (Lorenzen et al. 2010).

3.2. European EAZA Management of the Selected Taxa

The variability of the European EAZA populations of the selected spiral-horned antelope species and subspecies is displayed in Table 1. The population sizes range from 82 in lesser kudu to 457 in sitatunga, and the number of zoos keeping individuals of the studied taxa varies from 9 in lesser kudu to 61 in common eland (Species360 2022). The two taxa with the most severe conservation statuses, mountain bongo and lesser kudu, show the highest percentage of pedigree known, and at least partial ancestry is determinable in all of the individuals currently present in the European EAZA institutions (Davis & Humphreys 2022; Species360 2022; Steck 2022). When considering the percentage of pedigree known, sitatunga and greater kudu are found on the polar opposite side of the spectrum (Jebram 2012; Zwanzger 2023; personal communication), nevertheless, at least some information about the ancestry is available for more than 95% of the current European EAZA stock of greater kudu (Species360 2022). The number of founders is equally variable, ranging from 13 in nyala (Nolasco 2019) to 33 in mountain bongo (Davis & Humphreys 2022). Although all of the taxa are mostly treated on an individual level, herd management is still applied in sitatunga and common eland (Species360 2022). Similarly, these species are the only two of the six studied taxa without a regularly published studbook, which limits the amount of accessible information.

Table 1: Overview of management of the European EAZA populations

	MOUNTAIN BONGO	NYALA	SITATUNGA	LESSER KUDU	GREATER KUDU	COMMON ELAND
number of animals[1] (31/12/2022)	167 (57.109.1)	211 (70.140.1)	457 (102.330.25)	82 (29.52.1)	186 (54.132.0)	455 (138.302.15)
number of zoos (31/12/2022)	49	33	55	9	32	61
studbook	YES	YES	NO	YES	YES	NO
% pedigree known	97% (2021)	26% (2018)	~45% (2023)	97.6% (2021)	26.4% (2011)	unknown
% of current stock with at least partially known ancestry	100%	92.4%	73.1%	100%	95.2%	81.5%
founders	33 (2021)	13 (2018)	25 (2003)	24 (2021)	24-25 (2011)	unknown
herd management[2]	NO	NO	YES	NO	NO	YES
IUCN conservation status[3]	CR	LC	LC	NT	LC	LC

[1] sex ratio is provided in the format males.females.unknown

[2] no = all animals are managed on individual level; yes = some institutions still apply herd management

[3] CR = Critically Endangered; LC = Least Concern; NT = Near Threatened

Sources of data: Zwanzger (2003; unpublished); Jebram 2012; IUCN SSC Antelope Specialist Group (2016b,c,d,e); IUCN Antelope Specialist Group (2017); Nolasco (2019); IUCN SSC Antelope Specialist Group (2020); Davis & Humphreys (2022); Species360 (2022); Steck (2022); Zwanzger (2023; personal communication)

4. Methods

4.1. Sample Collection

Tissue, hair, or blood samples were obtained from eighteen European zoos distributed across six countries, and additional samples of common elands were collected at the University Farm Estate Lány of the Czech University of Life Sciences Prague. The list of the sample collection localities is provided in Table 15 in Appendix 1, including both the official names and their abbreviated versions used throughout this thesis. The samples were collected by Ing. Kateřina Štochlová. For the analysis of mitochondrial DNA, several older samples sourced from European zoos were used, and the institution of origin of most of them is unknown.

4.2. Laboratory Work

The samples were processed in the Laboratory of Molecular Genetics (Faculty of Tropical AgriSciences, Czech University of Life Sciences Prague) by Ing. Kateřina Štochlová. Genomic DNA was extracted using the DNeasy Blood & Tissue Kit (Qiagen) for tissue and hair samples and the Genomic Blood/Cultured Cell DNA Mini Kit (Geneaid) for blood samples, following the protocols enclosed by the manufacturers.

Polymerase chain reaction (PCR) was performed in the T100 Thermal Cycler (BIO-RAD) in order to amplify ten selected microsatellite loci, which are listed in Table 2. A reaction mixture of 10 μl was prepared for each sample, using 5 μl of Type-it Multiplex PCR Master Mix (Qiagen), 3 μl of RNase-free water, 1 μl of the primer mix, and 1 μl of the extracted DNA. Two reactions were performed for every sample, one with each of the two primer mixes, following the thermal protocol presented in Table 3. The fragment analysis was done in the Laboratory of Molecular Genetics (Faculty of Environmental Sciences, Czech University of Life Sciences Prague), using a reaction mixture of 8.5 μl of formamide, 0.5 μl of GeneScan 500 LIZ dye Size Standard (Applied Biosystems), and 1 μl of PCR product.

Table 2: Primers used for microsatellite DNA analysis

PRIMER MIX 5			
MARKER	**FLUORESCENT DYE**	**DETECTED SIZE IN BASE PAIRS (bp)**	**REFERENCE**
CSRM60	FAM	83-129	Moore et al. 1994
ETH225	FAM	131-165	Steffen et al. 1993
ETH10	FAM	200-226	Toldo et al. 1993
BL42	FAM	279-315	Bishop et al. 1994
X80214	VIC	203-239	Pépin et al. 1995
BRR	NED	233-261	Bishop et al. 1994
PRIMER MIX 6			
MARKER	**FLUORESCENT DYE**	**DETECTED SIZE IN BASE PAIRS (bp)**	**REFERENCE**
INRA107	FAM	148-180	Vaiman et al. 1994
BM4505	FAM	239-291	Beja-Pereira et al. 2004
SPS113	PET	127-155	Moore et al. 1994
CSSM42	PET	156-222	Moore et al. 1994

Table 3: PCR protocol for amplification of microsatellites

step	temperature (°C)	duration (min)
1	95	5:00
2	95	0:30
3	60	1:30
4	72	0:30
5	go to step 2, 30×	
6	68	30:00
7	12	forever

The mitochondrial control region (D-loop) was amplified by PCR using a 25 μl reaction mixture consisting of 12.5 μl of PPP Master Mix (Top-Bio), 8.5 μl of RNase-free water, 1 μl of MT4 primer (Arnason et al. 1993), 1 μl of BT16168H primer (Simonsen et al. 1998), and 2 μl of the extracted DNA. The reaction was performed in the T100 Thermal Cycler (BIO-RAD) based on the protocol included in Table 4. The success of the amplification was verified by gel electrophoresis, and in the case of a positive result, the PCR product was purified using the Gel/PCR DNA Fragments Extraction Kit (Geneaid) according to the enclosed protocol. The sequencing analysis was run in the Laboratory of Molecular Genetics (Faculty of Environmental Sciences, Czech University of Life Sciences Prague).

Table 4: PCR protocol for amplification of control region

step	temperature (°C)	duration (min)
1	95	5:00
2	95	1:00
3	55	1:00
4	72	1:00
5	go to step 2, 29×	
6	72	10:00
7	12	forever

4.3. Microsatellite DNA

The obtained data were edited and binned in Geneious 10.2.6. (https://www.geneious.com), and individual genotypes were created. Genetic clustering was evaluated using STRUCTURE 2.3.4. (Pritchard et al. 2000) with burn-in period set to 500,000 and number of Markov chain Monte Carlo (MCMC) repetitions after burn-in to 1,000,000. The analysis was run for K ranging from 1 to 10, with each performed in five iterations. The obtained results were processed in Structure Selector (Li & Liu 2018), where the highest-supported K was determined by the Delta K analysis (Evanno et al. 2005), and summarized results for the selected K were extracted. In addition to that, principal coordinates analysis (PCoA) was performed in GenAlEx 6.503 (Peakall & Smouse 2006; Peakall & Smouse 2012) to further investigate the genetic differentiation of the populations. For the taxa showing presence of genetic sub-structuring, the STRUCURE analysis was performed separately, with the parameters modified to a burn-in period of 200,000 and 800,000 MCMC repetitions after burn-in, with the K ranging from 1 to 7, each performed in five iterations.

Descriptive parameters of nucleic diversity, such as number of alleles (N_a), number of effective alleles (N_e), observed heterozygosity (H_o), and expected heterozygosity (H_e) were computed using GenAlEx 6.503 (Peakall & Smouse 2006; Peakall & Smouse 2012). In addition to that, inbreeding coefficient (F_{is}), including the 95% confidence interval (CI), was calculated in Genetix 4.05 (Belkhir et al. 2004). The same software was used to measure deviation from Hardy-Weinberg equilibrium by heterozygote deficit and excess, applying the 0.05 level of significance.

4.4. Mitochondrial DNA

Maternal lineage of each living individual was reconstructed, following the maternal ancestry in the ZIMS Species 360 database (2022) or in the studbooks, when accessible. The EAZA studbook numbers were used to designate the female ancestors, except for the individuals where this identifier was unavailable, and GAN (Global Accession Number) or local identification number was used instead. If the wild female founder of the maternal lineage could not be determined, the most ancient captive female ancestor was used. In the cases where even information about the mothers of the animals was missing, the maternal lineage was labeled as unknown. The maximum number of wild female founders of the European EAZA population was determined.

The mitochondrial control region sequences were trimmed and further edited using Geneious 10.2.6. (https://www.geneious.com). Additional sequences of the same region of mitochondrial DNA were downloaded from the NCBI Nucleotide database, and their country of origin was determined, when feasible. The accession numbers of these sequences were used in phylogenetic trees, and their detailed list is provided in Appendix 2. For each of the species or subspecies, the sequences were further analyzed separately. They were imported into the BioEdit Sequence Alignment Editor 7.2.5 (Hall 1999), where they were aligned using the ClustalW multiple alignment (Thompson et al. 1994) and trimmed to reach an equal length. The number of haplotypes was determined using DnaSP 6.12.03 (Rozas et al. 2017), and the original file with the sequences was consequently reduced to contain only one sequence of each haplotype.

The edited file was imported into MEGA11 (Tamura et al. 2021), where the neighbor-joining phylogenetic tree was constructed using the p-distance method as the number of base differences per site. The bootstrap values, which assess the reliability of the branching pattern, were reached by generating of 500 replicates, and they were displayed within the phylogenetic trees if they reached or exceeded 50. The phylogenetic tree was visualized and graphically edited using FigTree 1.4.4. (Rambaut 2006).

Genetic distances among the sequences, calculated as base differences per site using the p-distance model, were equally exported from MEGA11 (Tamura et al. 2021). The average genetic distance for the entire dataset was computed, as well as mean inter- and

intra-group distances if the presence of different clades was found in the phylogenetic tree analysis.

PopART 1.7 (Leigh & Bryant 2015) was used to create a transitive consistency score (TCS) haplotype network (Clement et al. 2002) for each of the analyzed taxa, with different colors representing different countries of origin of the samples. The network was redrawn, preserving the proportionality of the length of lines separating the haplotypes to the number of mutation steps differentiating the haplotypes. The cases where this proportionality could not be maintained were marked in the haplotype network by red color of the lines.

5. Results

5.1. Microsatellite DNA

A total of 179 samples were used in the microsatellite DNA analysis, with the overview of the number of individuals and institutions included in the dataset being presented in Table 5, and more details included in Table 16 in Appendix 3. Clear distinction of all of the six studied taxa was revealed by both STRUCTURE analysis and PCoA. The results of STRUCTURE for K=9, the highest supported number of clusters by the Delta K analysis, are visualized in Figure 7. Even though the PCoA for the two main axes only revealed the uniqueness of the nyala and lesser kudu populations, the distinction of all taxa was observed when the third axis was included, which is apparent from Figures 8 and 9.

Table 5: Samples used for microsatellite DNA analysis

	SAMPLES	INSTITUTIONS
MOUNTAIN BONGO	10	3
NYALA	63	9
SITATUNGA	24	6
LESSER KUDU	38	3
GREATER KUDU	17	2
COMMON ELAND	27	3

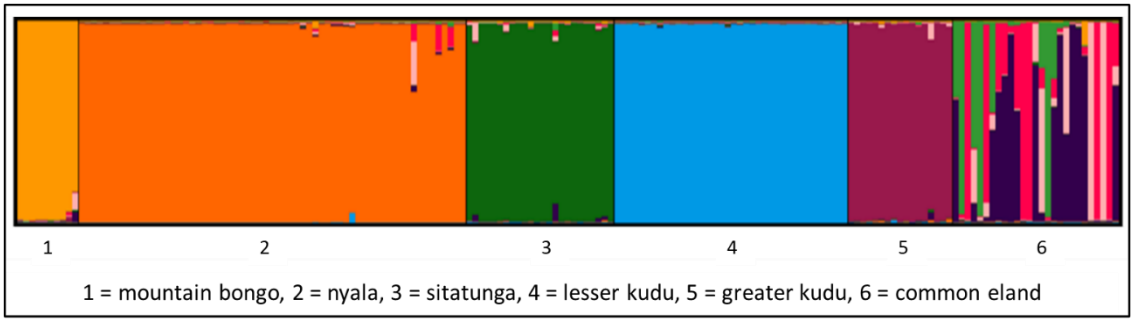

Figure 7: Bayesian clustering analysis with nine clusters shown; created in STRUCTURE. Putative origin of the groups is based on species determination by mitochondrial DNA analysis.

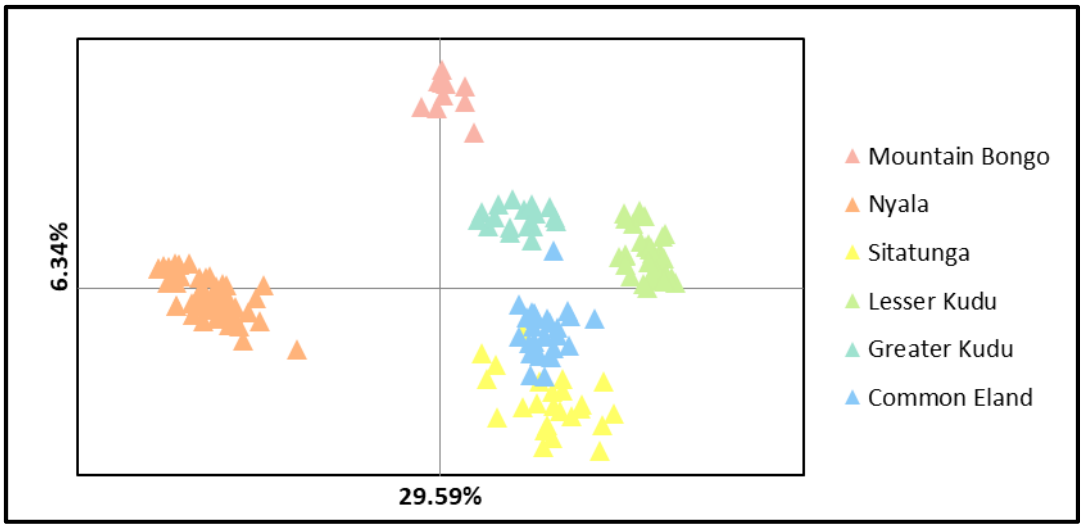

Figure 8: PCoA analysis for axes 1 and 3; created in GenAlEx

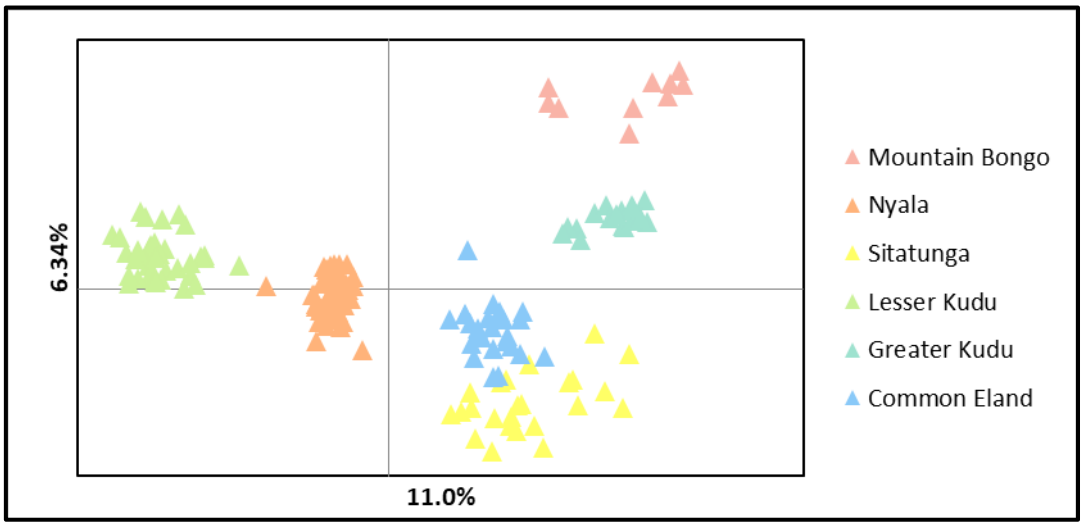

Figure 9: PCoA analysis for axes 2 and 3; created in GenAlEx

Further structuring was only found in the common eland population, and consequently, a separate STRUCTURE analysis was performed for this species. Based on the Delta K analysis, the highest support was attributed to K=3, the results of which are presented in Figure 10.

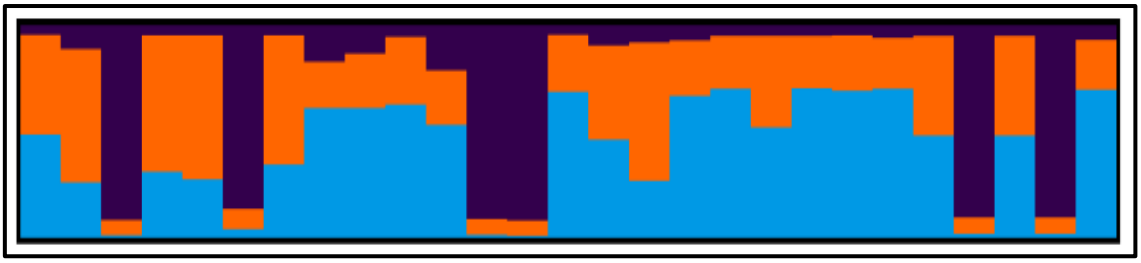

Figure 10: Bayesian clustering analysis results for K=3 in common elands; created in STRUCTURE

5.1.1. Descriptive Parameters of Nuclear Diversity

The overview of number of alleles per taxon and locus, presented in Table 6, revealed all taxa to be polymorphic in every amplified locus, apart from the mountain bongo, where 40% of the selected loci were monomorphic. On the contrary, the two highest numbers of alleles per locus were observed in common eland and greater kudu, reaching 12 and 10, respectively.

Table 6: Overview of number of alleles per population and locus; computed by GenAlEx

	MOUNTAIN BONGO	NYALA	SITATUNGA	LESSER KUDU	GREATER KUDU	COMMON ELAND
CSRM60	4	-	4	10	5	12
ETH225	1	2	7	2	5	9
ETH10	2	2	6	5	2	6
BL42	3	2	4	6	2	7
X80214	1	2	8	7	9	9
BRR	2	4	4	3	6	6
INRA107	1	5	3	4	5	7
BM4505	4	2	4	4	6	7
SPS113	2	5	4	3	6	6
CSSM42	1	6	4	-	5	8

Other parameters for the six populations are shown in Table 7. The mean number of alleles, as well as the number of effective alleles, was the lowest in mountain bongo, and the highest in common eland. The other four taxa showed values of both parameters in between the two extremes and quite similar to one another. However, while nyala had a higher mean number of alleles per locus than mountain bongo, the number of effective alleles was comparable. Similarly, although the sitatunga had a higher average number of alleles per locus than lesser kudu, the situation was reversed when considering the effective number of alleles.

Similarly to the previously mentioned parameters, the values of observed and expected heterozygosity were the largest in common eland and the smallest in mountain bongo. Four species (nyala, lesser kudu, greater kudu, common eland) showed higher observed heterozygosity compared to the expected one, while the populations of the other two taxa (mountain bongo, sitatunga) contained fewer heterozygotes than would be expected. Nevertheless, the deviation from the Hardy-Weinberg equilibrium was only significant in mountain bongo and common eland, as may be seen in Table 8. These results are equally

mirrored by the inbreeding coefficient, with the former of the two groups reaching negative values and the latter attaining positive values. The highest degree of inbreeding was observed in mountain bongo, whereas the nyala population showed the lowest value of this parameter.

Table 7: Other descriptive genetic parameters; computed by GenAlEx and Genetix

	MOUNTAIN BONGO	NYALA	SITATUNGA	LESSER KUDU	GREATER KUDU	COMMON ELAND
N_a	2.100	3.000	4.800	4.400	5.100	7.700
N_e	1.481	1.504	2.889	2.993	3.280	4.804
H_o	0.164	0.363	0.612	0.603	0.676	0.842
H_e	0.208	0.322	0.618	0.580	0.635	0.786
F_{is}	0.260	-0.119	0.034	-0.026	-0.033	-0.051
95% CI low	-0.059	-0.189	-0.111	-0.120	-0.176	-0.127
95% CI high	0.441	-0.063	0.118	0.046	0.027	-0.018

N_a = number of alleles; N_e = number of effective alleles; H_o = observed heterozygosity; H_e = expected heterozygosity; F_{is} = inbreeding coefficient; CI = confidence interval

Table 8: Hardy-Weinberg equilibrium; computed by Genetix

	HETEROZYGOTE DEFICIT (p-value)	HETEROZYGOTE EXCESS (p-value)
MOUNTAIN BONGO	0.0285*	0.9730
NYALA	0.1611	0.8389
SITATUNGA	0.1257	0.8743
LESSER KUDU	0.8546	0.1454
GREATER KUDU	0.4543	0.5457
COMMON ELAND	0.9793	0.0207*

*indicates significant p-values at 0.05 level of significance

5.2. Mitochondrial DNA

5.2.1. Mountain Bongo

According to the annually published studbook report (Davis & Humphreys 2022) and the institutional data submitted to the Species360 (2022) database, maternal ancestry of 67 (40.1%) of the living individuals could be tracked back to their female wild founders, which were three animals imported from Kenya. The remaining 100 individuals (59.9%) are descendants of seven different captive females of unknown origin. Consequently, the current European EAZA mountain bongo population originates from the maximum of ten

wild female founders. The overview of the situation is provided in Table 17 in Appendix 4.

The total of eight samples were used for the mitochondrial DNA analysis, with six of them corresponding to five of the potential maternal lineages, and the remaining two being from mountain bongos of unknown identity and origin. Although the analyzed samples do not encompass all putative maternal lineages, the ones that are included in the dataset represent 88% of the current European EAZA mountain bongo population. Additional information about the samples is included in Table 9. They were analyzed along with three sequences sourced from the NCBI Nucleotide database, two of which were of individuals from Kenya, and one was of a lowland bongo specimen from Congo.

Table 9: Mountain bongo samples used for mitochondrial DNA analysis

	GAN	INSTITUTION OF SAMPLE COLLECTION	MATERNAL LINEAGE
BO01	QCP12-00364	Dvůr Králové	165
BO02	27401447	Dvůr Králové	193
BO03	QCP15-02910	Dvůr Králové	185 (Kenya, Naiwasha)
BO04	MIG12-29523597	Peaugres	313
BO05	SGL16-02840	Gaia Zoo	3 (Kenya)
BO06	GNV14-00269	Knowsley	3 (Kenya)
BO07	unknown	unknown	unknown
BO08	unknown	unknown	unknown

After trimming of the sequences to an equal length of 392 base pairs, the DnaSP software revealed the presence of three haplotypes. All of the analyzed samples were found to be of one haplotype, identical to the EU040246.1 sequence from Kenya. The genetic distance between the two Kenyan bongo haplotypes was 0.00255, and it ranged from 0.51 to 0.54 between the mountain bongo samples from Kenya and the lowland bongo specimen from Congo. The neighbor-joining tree is presented in Figure 11, where the analyzed samples are indicated by a red underline. Same structuring, showing the difference of a single mutation step between the two Kenyan haplotypes, was revealed by the haplotype network, depicted in Figure 12. The lowland bongo sample was omitted from the analysis.

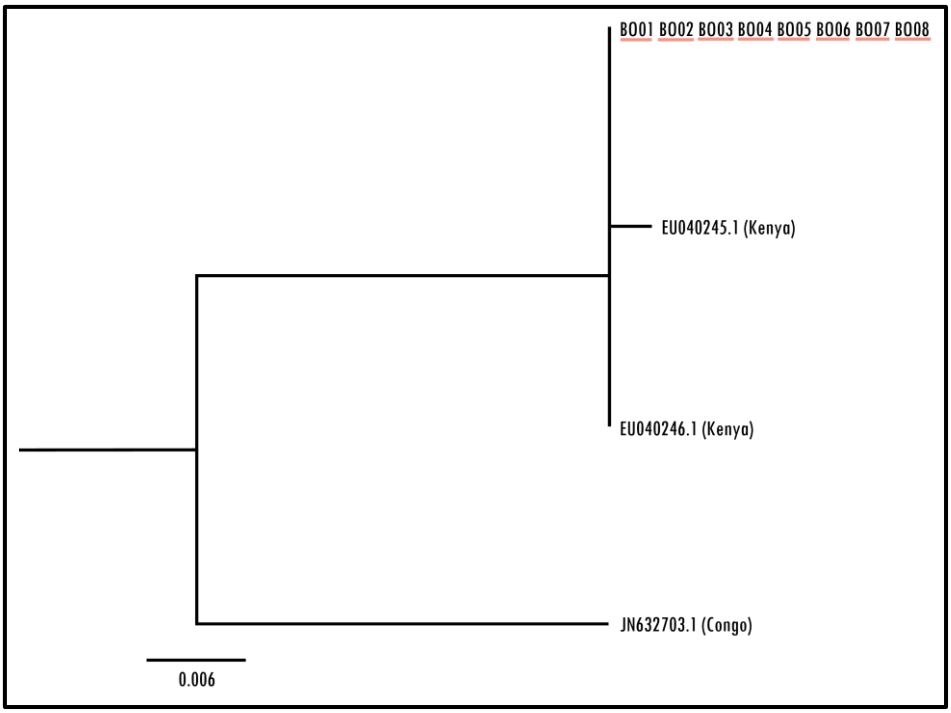

Figure 11: Neighbor-joining tree for mountain bongo; created by MEGA11 and FigTree (redrawn)

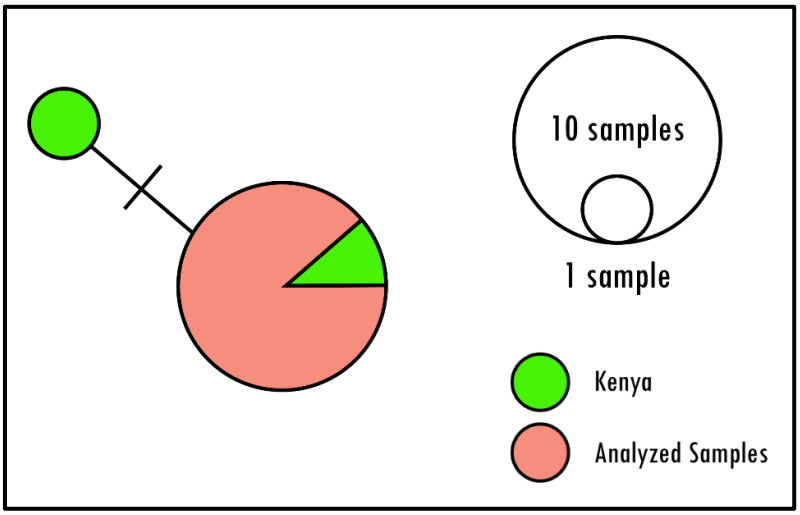

Figure 12: TCS haplotype network for mountain bongo; created by PopART (redrawn)

5.2.2. Nyala

Using a combination of data from the studbook report (Nolasco 2019) and the information submitted to the Species360 (2022) database by institutions, maternal ancestry of 78 (37%) of living animals could be reconstructed until their female wild founders, which were two nyalas from South Africa, were reached. Some degree of maternal ancestry was

determined in further 117 individuals (55.5%), who are descendants of twelve different captive females of unknown origin. However, no female ancestors are known for the remaining 16 animals (7.6%). The maximum number of wild female founders of the European EAZA population could thus reach 30, nevertheless this conclusion would require each of the nyalas with incomplete ancestry to represent a separate maternal lineage, which is highly unlikely. An overview of maternal lineages is presented in Table 18 in Appendix 4, where every animal with unidentified ancestry is included in the "unknown" group.

Eight of the nine samples used in the mitochondrial DNA analysis embody seven of the putative maternal lineages, and the nineth sample could not be assigned to any of the thirty hypothetical lineages. However, since the maternal ancestry of this individual could not be determined in its entirety, similarly to many others, no definitive conclusion about the uniqueness of this sample may be made. The maternal lineages encompassed in the dataset represent approximately 65% of the current European EAZA population of nyalas. Table 10 contains supplementary information about the analyzed samples. Additional 20 sequences retrieved from the NCBI Nucleotide database were used for the mitochondrial DNA analysis, originating from Malawi, Mozambique, South Africa, and Zimbabwe.

Table 10: Nyala samples used for mitochondrial DNA analysis

	GAN	INSTITUTION OF SAMPLE COLLECTION	MATERNAL LINEAGE
NY01	QCP17-03707	Dvůr Králové	344
NY02	QCP17-03830	Dvůr Králové	297
NY03	MIG12-29264407	Hannover	6 (South Africa)
NY04	MIG12-29038684	Vienna	530
NY05	MIG12-29194337	Vienna	126
NY06	14940854	Edinburgh	516
NY07	26774855	Edinburgh	3
NY08	FMY16-01289	Jihlava	6 (South Africa)
NY09	MIG12-29523411	Marwell	195

All of the sequences were trimmed to an equal length of 357 base pairs, and the total of ten haplotypes was revealed by the analysis done in the DnaSP software. Although none of the analyzed samples were identical to the sequences from the NCBI Nucleotide database, some of them shared a haplotype among each other, and the nine analyzed samples were consequently reduced to four haplotypes.

The neighbor-joining tree, presented in Figure 13 with the analyzed samples indicated by an orange underline, showed two clusters, one including sequences from Zimbabwe and

South Africa and the other comprising specimens from Mozambique and Malawi. The average genetic distance between samples from the same cluster was 0.011 (varying between 0.0028 and 0.02) while the mean value for sample pairs from different clusters was 0.017 (ranging from 0.01 to 0.027). Three of the four haplotypes containing the analyzed samples clustered with the clade from Zimbabwe and South Africa, whereas solely one haplotype was associated with the group from Mozambique and Malawi.

The only two samples with fully known maternal ancestry, NY03 and NY08, grouped with the cluster from South Africa and Zimbabwe, which was consistent with the South African origin of their female wild founders. The sample pairs collected in Dvůr Králové and Vienna Zoo both consisted of one sample from each of the main clades. On the contrary, the dyad of samples from Edinburgh Zoo was only associated with the South African and Zimbabwean group.

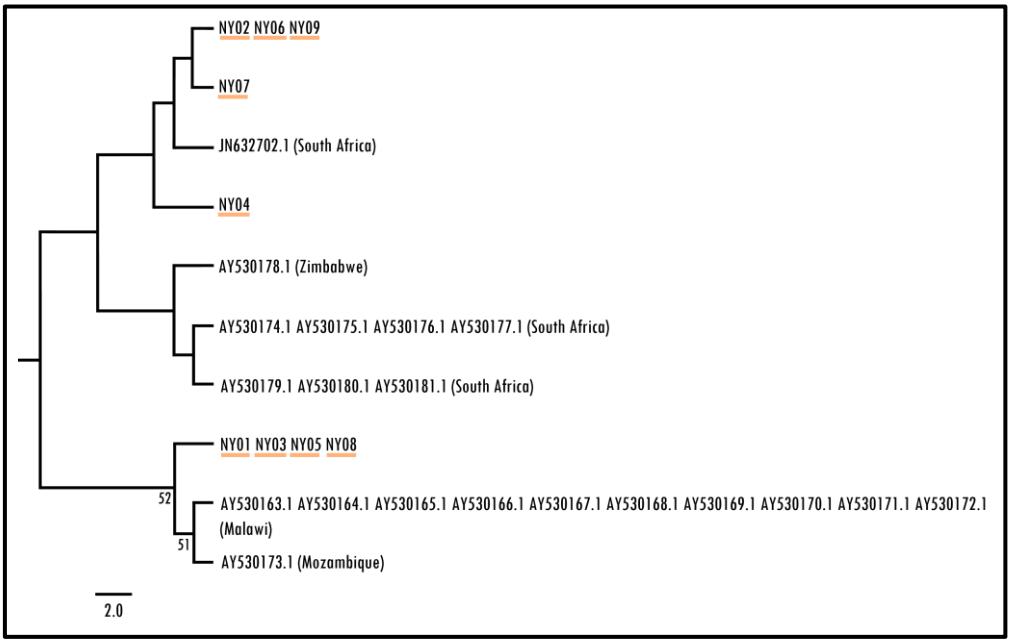

Figure 13: Neighbor-joining tree for nyala; created by MEGA11 and FigTree (redrawn)

The haplotype network, shown in Figure 14, revealed a relatively shallow structure with only small differentiation among the haplotypes and the number of mutation steps between neighboring haplotypes attaining between one and four. Consequently, the network shows no clear distinction of the two clusters displayed in the neighbor-joining tree, which is consistent with the low values of genetic distances among the samples.

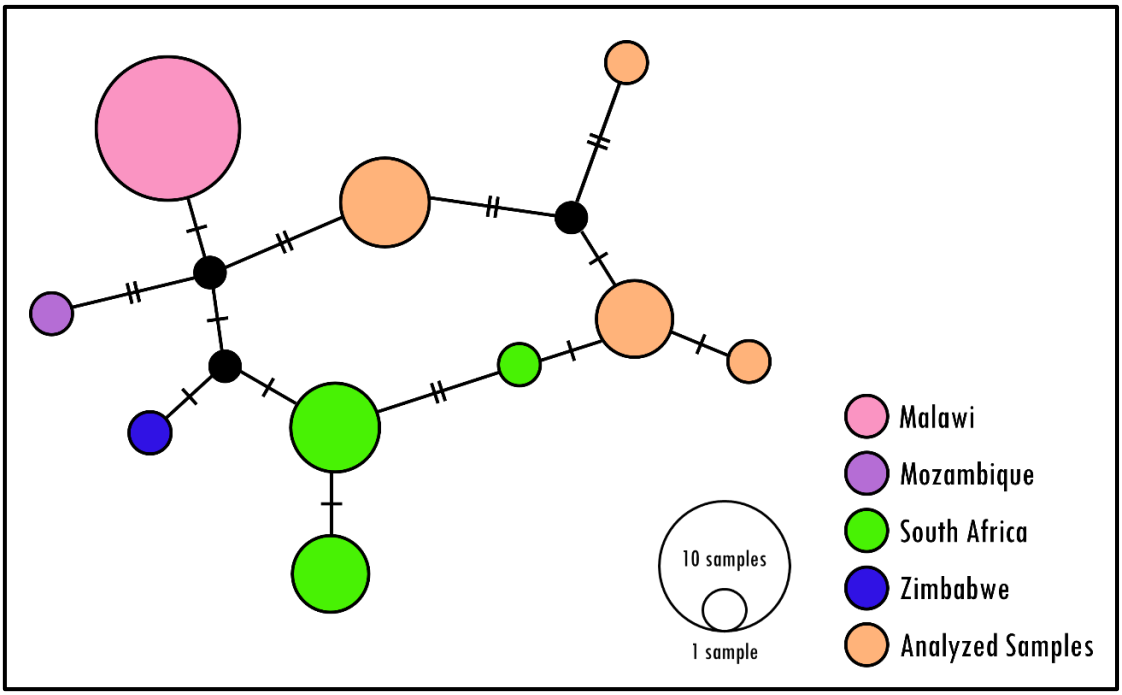

Figure 14: TCS haplotype network for nyala; created by PopART (redrawn)

5.2.3. Sitatunga

Using the institutional information submitted to the Species360 database (2022) by individual zoos, complete maternal lineage until the female wild founder could not be reconstructed in any of the 457 living animals. Although some degree of maternal ancestry could be determined in the case of 334 individuals (73.1%), which are descendants of 66 different captive female sitatungas of unknown origin, no female ancestors of the remaining 123 animals (26.9%) are identifiable. In the highly improbable event of each of the individuals with incomplete ancestry representing a separate lineage, the current European EAZA population would have 189 wild female founders. Nonetheless, as this high value may be attributed to the large proportion of unknown pedigree, the actual number is expected to be much lower. The summary of maternal lineages is displayed in Table 19 in Appendix 4, with all animals with unidentified maternal history included in the "unknown" group.

A total of thirteen sitatunga samples were used in the mitochondrial DNA analysis, with six of them representing one potential maternal lineage each. The other seven samples were collected from unidentifiable individuals, and no information on their ancestry could thus be obtained. The six maternal lineages embodied in the dataset merely represent 14% of the present European EAZA population of sitatunga, which may be linked to the fact

that only three of the ten most prevalent lineages were included in the dataset. In addition to that, the large proportion of missing pedigree data led to the emergence of many apparent lineages that could likely have been reduced to a much lower number, had the complete information on ancestry been available, and the analyzed samples thus probably represent a higher proportion of the population than it seems. Further details about the samples are provided in Table 11. They were analyzed together with seven sequences from Gabon, Cameroon, and Republic of Congo, as well as one sequence of unknown origin, which were all sourced from the NCBI Nucleotide database.

Table 11: Sitatunga samples used for mitochondrial DNA analysis

	GAN	INSTITUTION OF SAMPLE COLLECTION	MATERNAL LINEAGE
SI01	BQK13-00885	Dvůr Králové	4833
SI02	QCP16-03324	Dvůr Králové	5047
SI03	QCP16-02968	Dvůr Králové	5246
SI04	MFK15-00173	Plaisance	4643
SI05	LJQ17-02042	Exmoor	7592
SI06	MIG12-28745736	Knowsley	6141
SI07	unknown	unknown	unknown
SI08	unknown	unknown	unknown
SI09	unknown	unknown	unknown
SI10	unknown	unknown	unknown
SI11	unknown	unknown	unknown
SI12	unknown	unknown	unknown
SI13	unknown	unknown	unknown

The length of the sequences was unified to 522 base pairs, and the analysis in the DnaSP software revealed the presence of 13 haplotypes. Even though none of the analyzed samples matched the sequences from the NCBI Nucleotide database, some of them shared a haplotype among one another, which clustered the thirteen samples into five haplotypes.

The neighbor-joining tree, displayed in Figure 15 with the analyzed samples indicated by a yellow underline, revealed two main groups, one containing samples from Western Gabon, and the other including sequences from Republic of Congo, Eastern Gabon, and Cameroon. While two of the haplotypes, containing one analyzed sample each, grouped with the Western Gabon clade, the remaining three haplotypes, encompassing 11 samples, clustered with the other clade. The samples collected from Dvůr Králové were represented in both of the main clusters. The average genetic distance within the clades was 0.023 (ranging from 0 to 0.043), while the mean value between the clades was 0.045 (varying between 0.036 and 0.054).

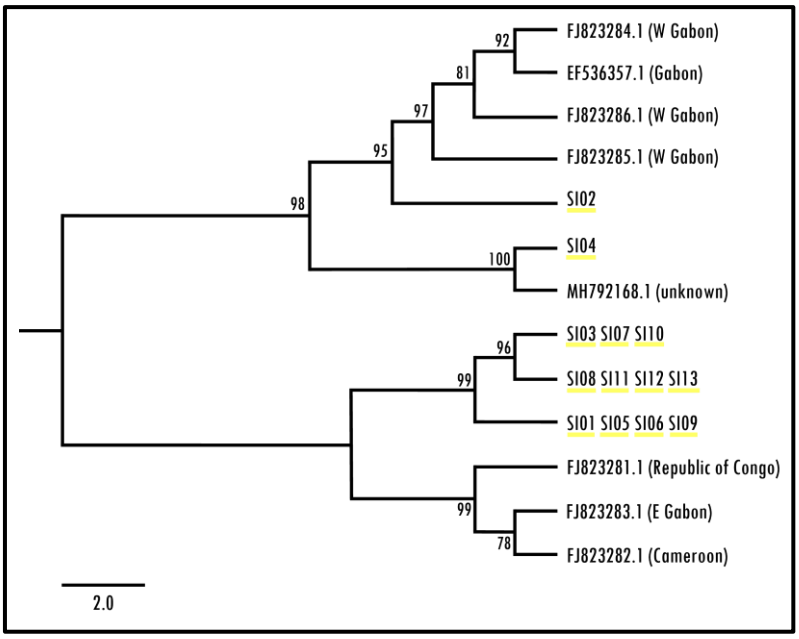

Figure 15: Neighbor-joining tree for sitatunga; created by MEGA11 and FigTree (redrawn)

Only twelve haplotypes were revealed in the haplotype network, displayed in Figure 16. The structuring is identical to the one shown in the neighbor-joining tree, and a high number of mutation steps separates the two main clusters, consistently with the high genetic distances between the clades. Furthermore, numerous cases where haplotypes from the same group differ by a larger number of mutation steps are observed, which is in agreement with the fact that some genetic distances within the clades are greater than those between the clades.

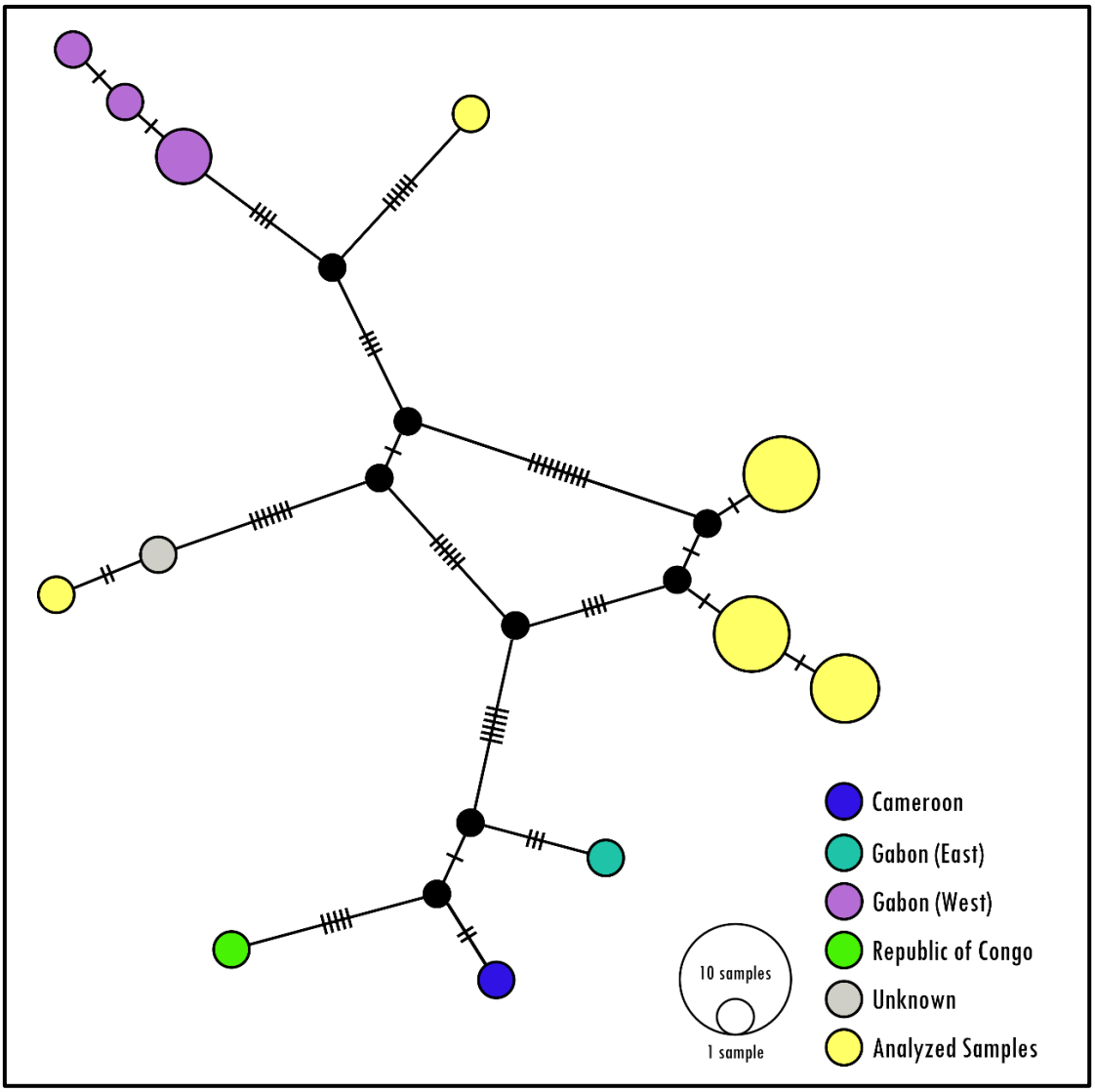

Figure 16: TCS haplotype network for sitatunga; created by PopART (redrawn)

5.2.4. Lesser Kudu

According to the annually published studbook report (Steck 2022) and the institutional data submitted to the Species360 (2022) database, maternal ancestry of 52 individuals (63.4%) could be tracked back to their female wild founders, which were four animals brought to Europe from Kenya. Other 29 lesser kudus (35.4%) are descendants of one captive female, whose mother was one of the wild founders from Kenya, even though her identity is undetermined. The ancestry of the remaining animal cannot be reconstructed in its entirety, and this individual thus represents a potentially separate lineage. The current European EAZA population of lesser kudu therefore originates from the

maximum of six wild female founders. The overview of maternal lineages is provided in Table 20 in Appendix 4.

Six of the eight samples used for the analysis of mitochondrial DNA were representative of four of the potential maternal lineages, and one other sample was of unknown origin. The eighth sample represented an entirely different maternal lineage, based on a female wild founder that was brought to Europe from Tanzania, which does not contribute to the current European EAZA population of lesser kudu, nevertheless, some of its members may still be kept in other institutions. The four maternal lineages included in the dataset represent 91.5% of the present European EAZA population. Additional information about the samples is provided in Table 12. They were analyzed alongside three sequences from Tanzanian animals, four sequences from wild Somalian lesser kudus that were captured and brought to the zoo population, and 46 sequences from captive lesser kudus, which were all extracted from the NCBI Nucleotide database. Due to the limited number of sequences from wild animals, maternal lineages of the captive specimens were identified, if possible, and indicated in the mitochondrial DNA analysis.

Table 12: Lesser kudu samples used for mitochondrial DNA analysis

	GAN	INSTITUTION OF SAMPLE COLLECTION	MATERNAL LINEAGE
LK01	QCP17-03628	Dvůr Králové	125
LK02	QCP16-03563	Dvůr Králové	103 (Kenya)
LK03	QCP16-03137	Dvůr Králové	106 (Kenya)
LK04	11104114	Hannover	26 (Kenya)
LK05	6797337	unknown	374 (Tanzania)
LK06	unknown	unknown	unknown
LK07	27401344	unknown	125
LK08	27401329	unknown	125

All of the used sequences were trimmed to an equal length of 522 base pairs, and the DnaSP software revealed the presence of 14 haplotypes. The eight analyzed samples were reduced to four haplotypes, three of them shared with sequences from the NCBI Nucleotide database and the remaining one being unique to the analyzed samples. The three animals sharing a maternal lineage based on pedigree data (LK01, LK07, and LK08) were separated into two haplotypes, however, this distinction may have been caused by the presence of missing data in some of the sequences, rather than their actual variability.

The neighbor-joining tree is displayed in Figure 17, with the analyzed samples highlighted by a green underline. No clear structuring was revealed, and sequences with

maternal lineages originating from different countries commonly clustered together, which implies low genetic diversity within the species. Although the Somalian animals did not share a haplotype with any other individual, they failed to cluster separately and were distributed throughout the neighbor-joining tree among the other sequences, which further supports the low genetic variability present within the species. This finding is equally mirrored in the small average genetic distance of 0.008 (ranging from 0 to 0.017) among the sequences.

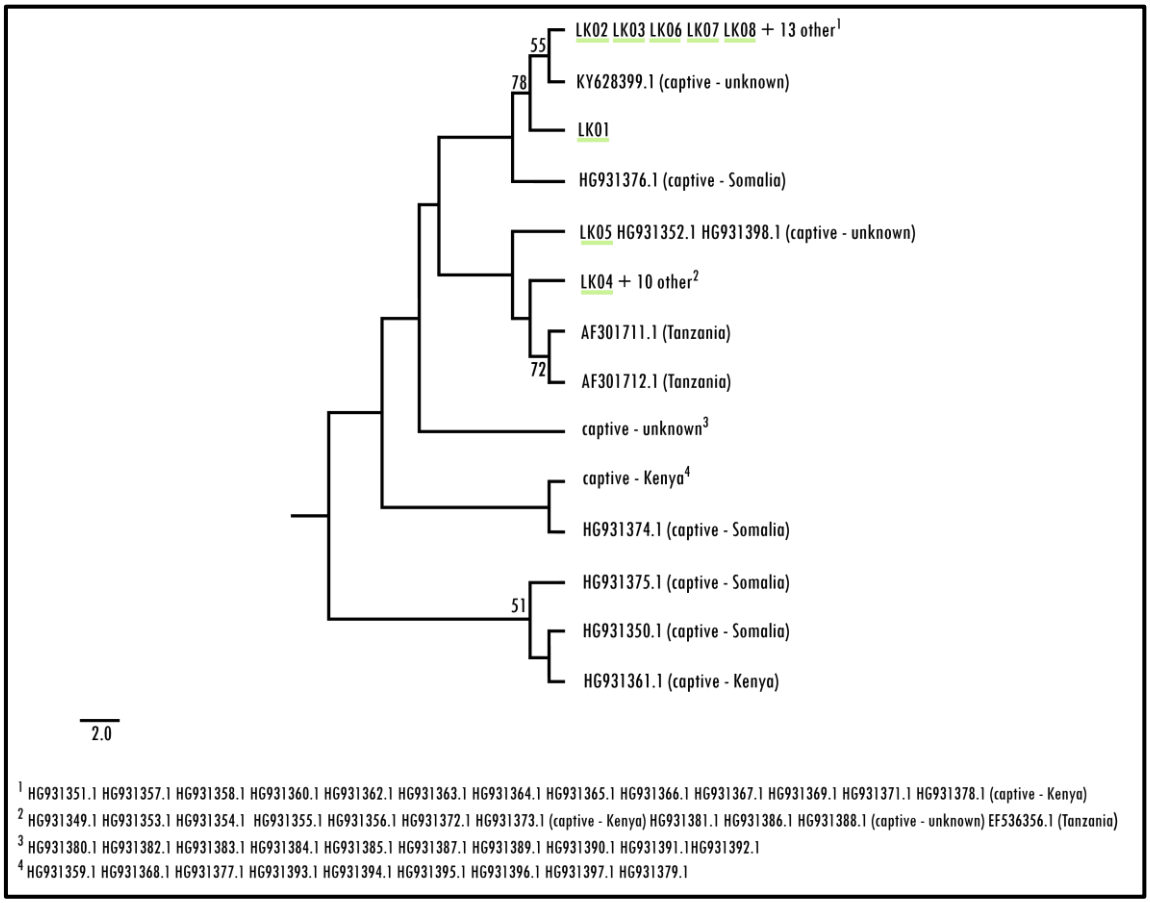

Figure 17: Neighbor-joining tree for lesser kudu; created by MEGA11 and FigTree (redrawn)

The haplotype network, depicted in Figure 18, revealed a similar structure, despite only showing the presence of ten distinct haplotypes. Identically to the neighbor-joining tree, the network showed no grouping according to geographic origin of the individuals. In addition to that, the maximum number of mutation steps between neighboring haplotypes reached the value of two, further supporting the low genetic differentiation within the

lesser kudu species. Contrary to the neighbor-joining tree, the analyzed samples sharing a maternal lineage were shown to share a haplotype in the haplotype network, which further supports the statement that their distinction in the neighbor-joining tree was associated with missing data in some of the sequences.

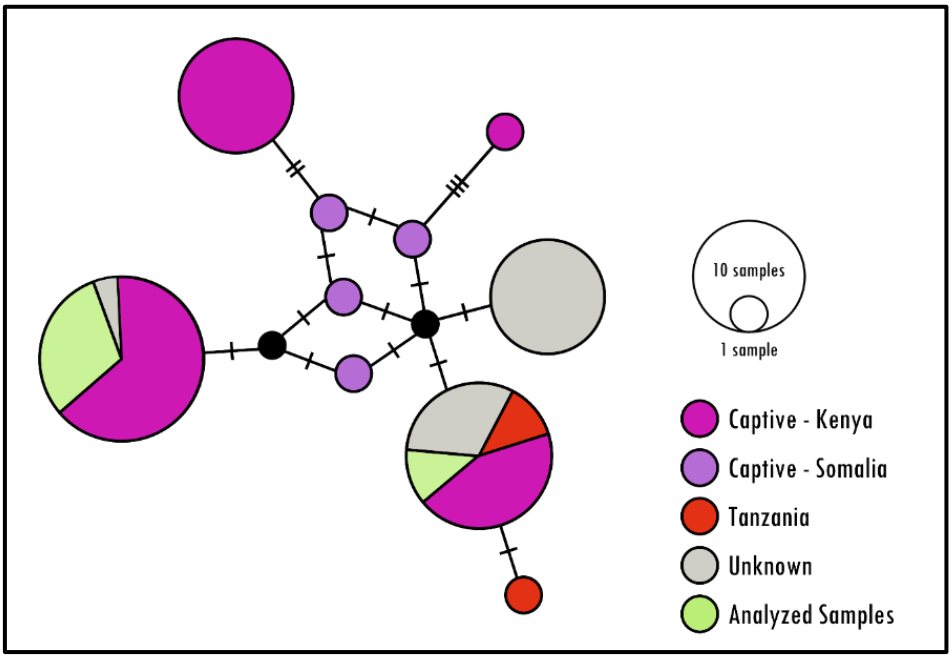

Figure 18: TCS haplotype network for lesser kudu; created by PopART (redrawn)

5.2.5. Greater Kudu

Using the institutional data submitted to the Species360 (2022) database, full maternal ancestry could be reconstructed in the case of 78 animals (41.9%), who are descendants of four wild females, two of which were captured in Namibia whereas the origin of the other two is unknown. Incomplete maternal lineage is determinable for further 99 individuals (53.2%), whose ancestry can be tracked back to 11 captive females of unidentified origin. No pedigree information is available for the remaining nine individuals (4.8%). Consequently, the current European EAZA greater kudu population could be based on up to 24 wild female founders, provided that each of the animals with unknown ancestry represented a separate maternal lineage. The overview of maternal lineages is presented in Table 21 in Appendix 4.

Ten of the 17 samples used in the analysis of mitochondrial DNA belong to five of the potential maternal lineages, and four other samples were of unknown origin. The remaining three samples could not be attributed to any of the 24 putative lineages, and could therefore represent separate lineages. However, as a portion of ancestry of the living

greater kudus remains unknown, no definite conclusion about the uniqueness of these samples can be made. The five maternal lineages represented in the dataset account for 78% of the present European EAZA population. Supplementary information about the analyzed samples is provided in Table 13. Further 114 sequences of individuals from Botswana, Namibia, South Africa, Zambia, and Zimbabwe, as well as two sequences of captive greater kudus of unknown origin, all sourced from the NCBI Nucleotide database, were used in the mitochondrial DNA analysis.

Table 13: Greater kudu samples used for mitochondrial DNA analysis

	GAN	INSTITUTION OF SAMPLE COLLECTION	MATERNAL LINEAGE
GK01	QCP14-02236	Dvůr Králové	615
GK02	YZQ14-00488	Dvůr Králové	5 (Namibia)
GK03	27410371	Dvůr Králové	483
GK04	27795027	Gaia Zoo	unknown
GK05	SGL13-00586	Gaia Zoo	483
GK06	JLD18-01465	Boissière	5 (Namibia)
GK07	QZZ17-01374	Munich	5 (Namibia)
GK08	YVK14-00344	Plzeň	615
GK09	YLH16-01790	Plzeň	383 (unknown)
GK10	22770409	La Palmyre	500
GK11	FVR12-00307	La Palmyre	500
GK12	PMQ14-00424	Sigean	unknown
GK13	22693089	unknown	154
GK14	27406598	unknown	175
GK15	unknown	unknown	unknown
GK16	unknown	unknown	unknown
GK17	22771214	unknown	LA PALMYR / F1

The used sequences were trimmed to an identical length of 416 base pairs, and the DnaSP software identified the presence of 99 haplotypes. The analyzed samples belonged to nine of these, nevertheless, none of them shared a haplotype with the sequences sourced from the NCBI Nucleotide database. Based on pedigree data, the dataset contained four groups of animals sharing a maternal lineage, but only two of these groups were shown in the haplotype analysis performed by the DnaSP software, however, this distinction may have been associated with the presence of missing data in the sequences.

The neighbor-joining tree, displayed in Figure 19 with the analyzed samples indicated by a turquoise underline, revealed the presence of two main clades. While one included sequences of animals originating from a wide range of countries from Tanzania to South Africa, the other only contained Namibian and South African individuals. All analyzed

samples clustered with the second clade, except for one animal of unknown origin, which associated with the first clade.

The average genetic distance among the sequences reached 0.0386, with mean value within the two main clades of 0.0181 (0.002-0.071) and that between the two main groups of 0.061 (0.04-0.098). However, the highest inter-clade distances were associated with the OK642774.1 sample from South Africa, which was separated from the rest of the clade by the average distance of 0.055. As a result, the removal of this sample reduced the inter-clade distances to the mean value of 0.0176 and the maximum was decreased to 0.049.

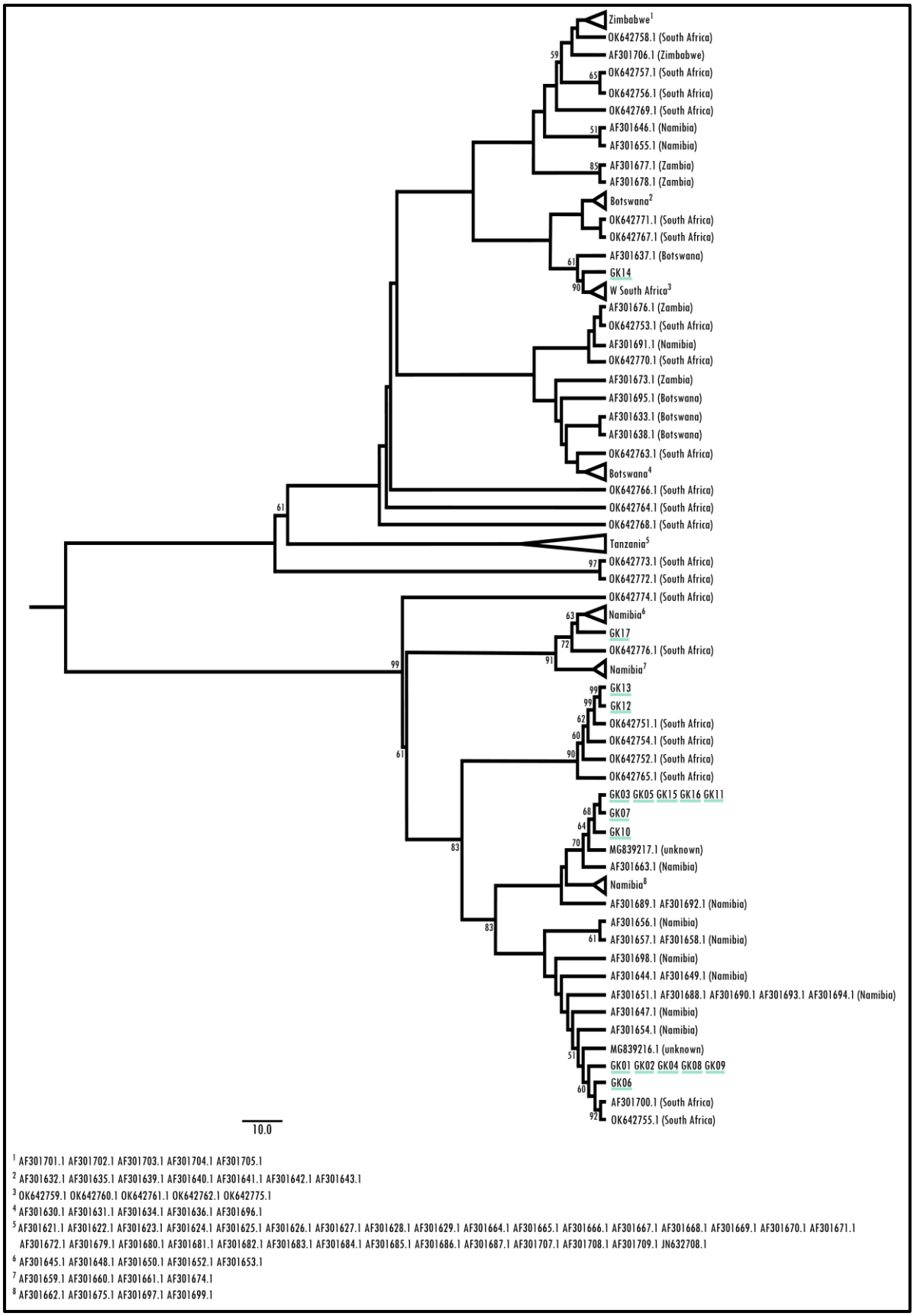

Figure 19: Neighbor-joining tree for greater kudu; created by MEGA11 and FigTree (redrawn)

The haplotype network, depicted in Figure 20, revealed the presence of 46 haplotypes. Despite the large difference in number of haplotypes, the structure of the network corresponded to that of the neighbor-joining tree in terms of geographic origin of the sequences. The four groups of individuals sharing maternal lineages determined from pedigree data were identified in the haplotype network, except for the animal GK07, which did not associate with the other two animals from its potential maternal lineage. Due to the complexity of the network, proportionality of the lines in Figure 20 could not always be maintained, and the cases where it was disrupted are indicated in red color.

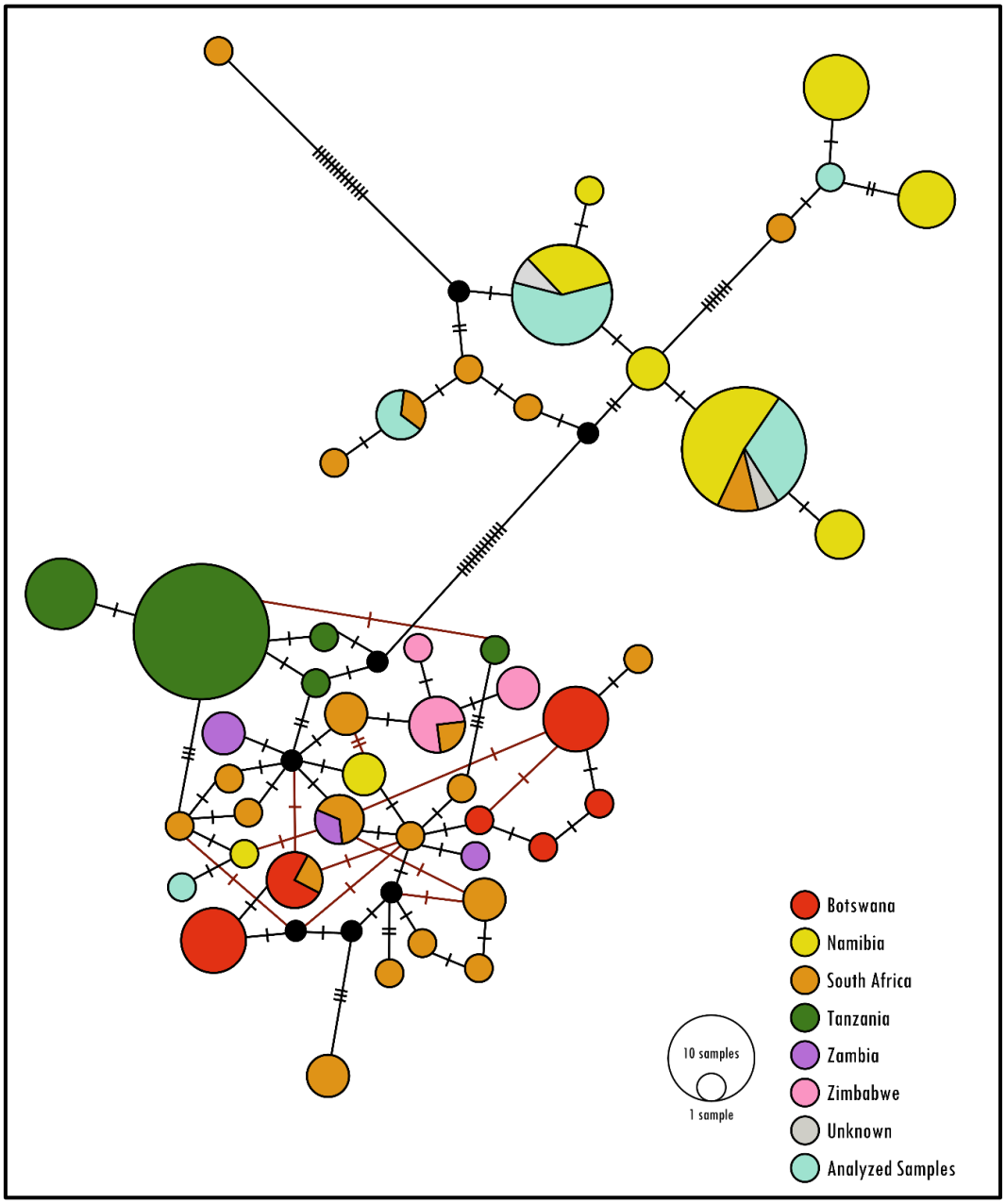

Figure 20: TCS haplotype network for greater kudu; created by PopART (redrawn)

5.2.6. Common Eland

Based on institutional data submitted to the Species360 (2022) database, complete maternal lineage until the female wild founder was impossible to reconstruct in any of the 455 living animals. Some degree of maternal ancestry could be tracked in 371 individuals (81.5%), nevertheless the quality of the pedigree data is low, which may be demonstrated by the fact that no ancestors other than the mothers could be identified in 78 cases (17.1%), and maternal lineages of further 89 individuals (19.6%) could only be followed until their grandmothers. In summary, the maximum of two generations of maternal ancestry could be determined for 251 common elands (55.2%) from the current European EAZA population. If every animal with partially known maternal ancestry represented a separate lineage, the total of wild female founders would reach 169 individuals, nonetheless, this number surely is an overestimation caused by the incompleteness of the pedigree data. The overview of maternal lineages is displayed in Table 22 in Appendix 4, where all animals of unidentified female ancestry are included in the "unknown" group.

Out of the 18 samples used for mitochondrial DNA analysis, ten could be attributed to one of the putative maternal lineages each. Another four samples do not belong to any of the lineages, and might thus potentially represent separate ones, nevertheless, no definitive conclusion about the uniqueness of these individuals may be made due to the large proportion of missing pedigree data. Ancestry of the remaining four animals is unknown. The ten maternal lineages contained in the dataset account for 16.9% of the current European EAZA population, however, the representativeness of the analyzed samples might actually be much higher since knowledge of full maternal ancestry would merge multiple lineages together. Table 14 provides more detailed information about the analyzed samples. Additional 128 sequences, sourced from the NCBI Nucleotide database, were used for the mitochondrial DNA analysis, some of them originating from wild common elands from Botswana, Ethiopia, Kenya, Namibia, South Africa, Tanzania, Uganda, Zambia, and Zimbabwe, while others were obtained from captive individuals of unknown origin.

Table 14: Common eland samples used for mitochondrial DNA analysis

	GAN	INSTITUTION OF SAMPLE COLLECTION	MATERNAL LINEAGE
CE01	QCP16-03525	Dvůr Králové	MIG12-29528527
CE02	QCP16-03288	Dvůr Králové	MIG12-29254642
CE03	QCP16-03577	Dvůr Králové	MIG12-29933742
CE04	QCP17-03618	Dvůr Králové	27399102
CE05	QCP12-00176	Dvůr Králové	20635495
CE06	QCP17-03848	Dvůr Králové	27409667
CE07	unknown	Hannover	unknown
CE08	BDP15-06353	Vienna	KWY16-00155
CE09	MLJ14-00757	Vienna	unknown
CE10	11425537	Munich	WUPPERTAL / 78000
CE11	SQY13-00381	Munich	MIG12-29421706
CE12	MRK14-00127	Plaisance	27700319
CE13	GWK15-01075	Knowsley	unknown
CE14	GWK15-01081	Knowsley	unknown
CE15	GWK17-01174	Knowsley	PLANCKNDL / M2430B
CE16	GWK16-01125	Knowsley	GWK15-01083
CE17	GWK17-01175	Knowsley	MIG12-29591594
CE18	MIG12-29949471	University Farm Estate Lány	MIG12-29949266

The used sequences were unified to an equal length of 397 base pairs, and the DnaSP software distinguished 111 haplotypes, with the analyzed samples belonging to ten of them. While two of these haplotypes were shared with the sequences from the NCBI Nucleotide database, eight of them were unique to the analyzed samples.

The neighbor-joining tree presented in Figure 21, where a blue underline indicates the analyzed samples, revealed the presence of two main clades. While one of the groups comprised sequences from eastern Africa (Ethiopia, Kenya, Tanzania, Uganda) and several animals from Zimbabwe, the other included sequences from southern Africa (Botswana, Namibia, South Africa, Zambia, Zimbabwe) and a few sequences from Tanzania. All of the analyzed samples associated with the eastern African clade, apart from one sequence of an individual sampled at the University Farm Estate Lány and currently residing in the Ostrava Zoological Garden and Botanical Park.

The average genetic distance reached 0.0388, with mean genetic distance attaining 0.0206 (0-0.081) within the two main clades and 0.0572 (0.007-0.083) between the clades. Nevertheless, the group of animals from Zimbabwe clustering with the eastern African sequences displayed a high distinction from the remainder of the clade, with mean genetic distance of these samples from the rest of the group reaching 0.0623. Similarly, the most basal individual in the southern African clade from Zimbabwe differed from the

remainder of the group by an average genetic distance of 0.0592. As a consequence, these animals increased the values of genetic distances within the clades, and they only reached an average of 0.0172 and a maximum of 0.38 when these individuals were removed from the calculation.

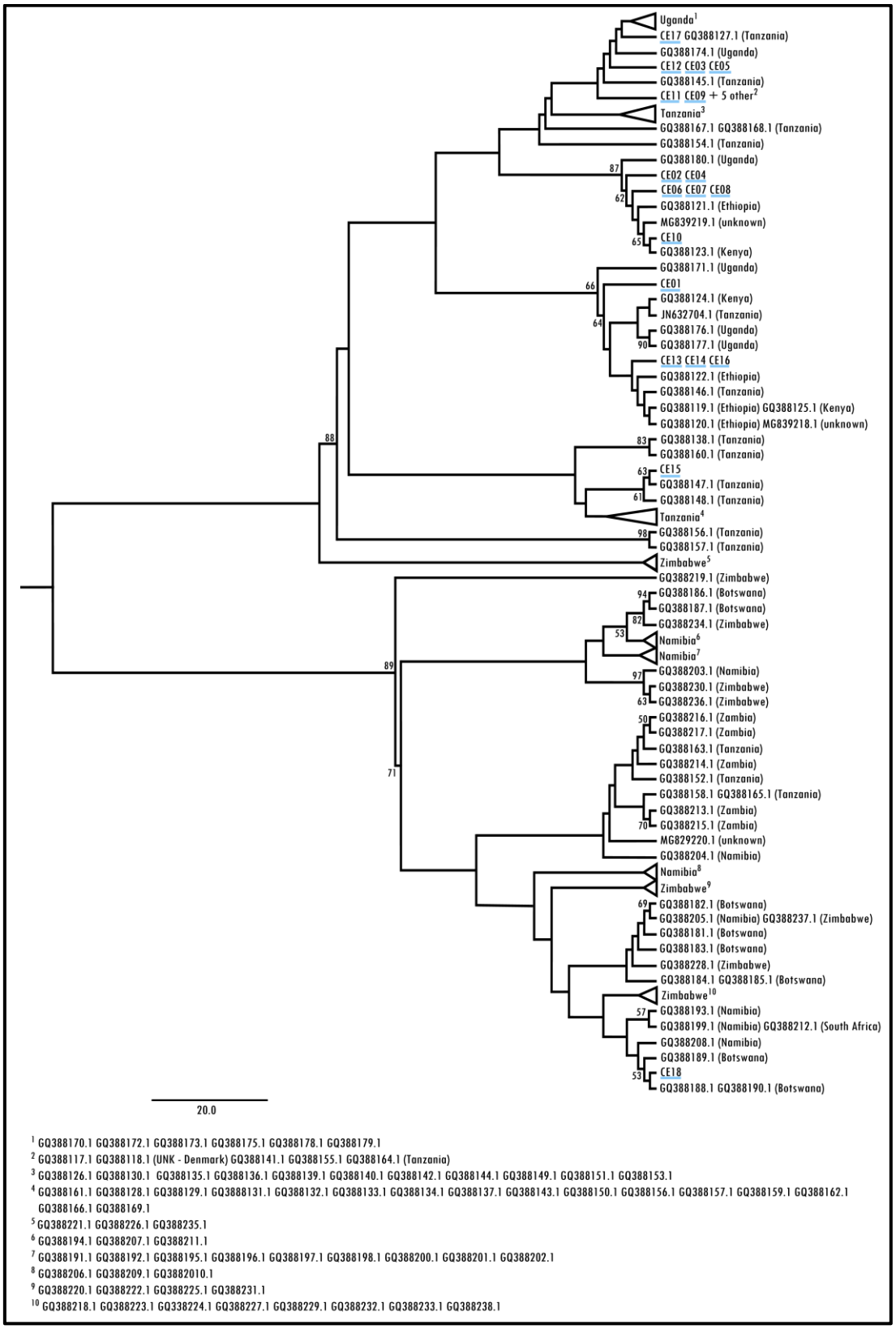

Figure 21: Neighbor-joining tree for common eland; created by MEGA11 and FigTree (redrawn)

The haplotype network, shown in Figure 22, revealed the presence of 50 haplotypes. Despite the large difference in number of haplotypes from the neighbor-joining tree, the structure with regards to geographic origin of the samples was similar. All of the analyzed samples except for the one from the University Farm Estate Lány clustered with the eastern African group. Identical to the neighbor-joining tree, some of the haplotypes from Zimbabwe had a unique position within the network, separated from the remainder of their respective clades by 11-12 mutation steps. On the contrary, other neighboring haplotypes within the clades were at most four mutation steps away from one another.

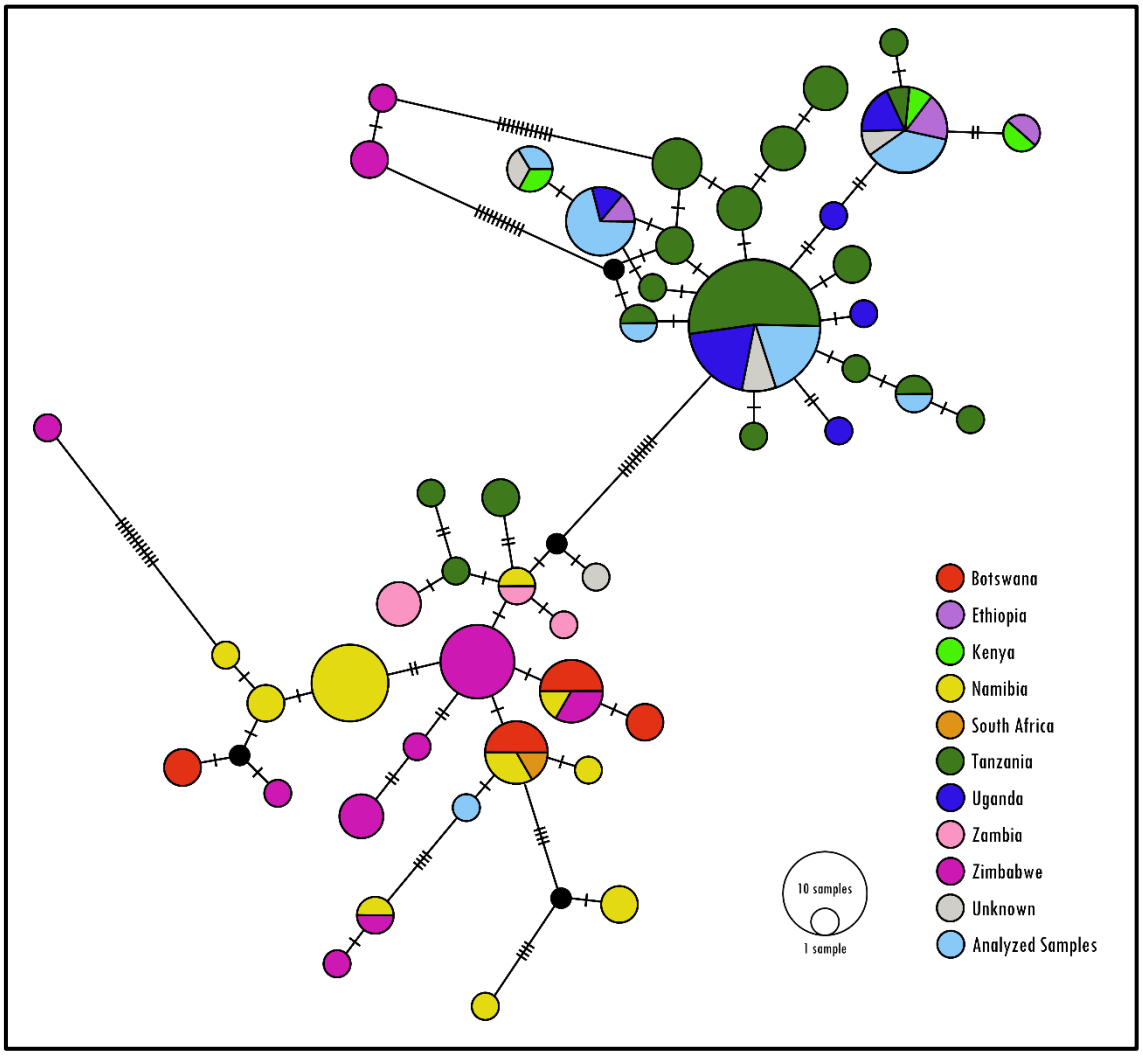

Figure 22: TCS haplotype network for common eland; created by PopART (redrawn)

6. Discussion

6.1. Management Impact on Genetic Parameters

6.1.1. Mountain Bongo

The low genetic diversity detected in mountain bongo based on microsatellite data is consistent with the findings of Combe et al. (2018), and it is probably linked to the reduced genetic variability of the wild population described by Faria et al. (2011), and the consequent limited diversity of the founders of the captive stock despite their relative numerousness. The impact of this factor could have been further aggravated by the fact that the European population of mountain bongo is solely based on individuals captured in one of the localities of wild occurrence, the Aberdare Mountains (Bishop et al. 2019), and if any additional variability was present uniquely in other parts of the distribution area, it is not represented in the captive population.

Inbreeding coefficient observed in the mountain bongo was extremely elevated compared to the other studied taxa, with a value exceeding that found in individuals produced by mating of parents with their offspring or reproduction of full siblings (Ballou et al. 2010), which might indicate that the current genetic management may not be effective enough to prevent mating of related animals. However, this situation possibly reflects the unfavorable state of the whole subspecies, limiting the opportunity for management to achieve a significant improvement. Inbreeding, as well as the impact of genetic drift (Ballou et al. 2010; Willoughby et al. 2015), may be mirrored in the observed deviation from the Hardy-Weinberg equilibrium and the detected significant excess of homozygotes. As inbred and homozygous populations might display reduced viability and greater susceptibility to extinction (Ballou et al. 2010; Ralls & Ballou 2013), these values are concerning for the further survival of the captive population. In addition to that, high inbreeding and low heterozygosity reduce the probability of successful reintroduction to the wild (Frankham et al. 2008; Ralls & Ballou et al. 2013), which is one of the conservation strategies proposed for the mountain bongo (O'Donoghue et al. 2017).

The limited genetic variability of the captive stock of mountain bongo might equally be linked to the low number of individuals, barely exceeding 150 animals (Davis &

Humphreys 2022), as small populations lose genetic diversity at a higher rate compared to bigger ones (Ballou et al. 2010; Willoughby et al. 2015). The situation could further be exacerbated by the continuous decline in the number of EAZA mountain bongo animals since the beginning of the 21st century, as well as the limited effective population size, only attaining less than a third of the actual number of individuals (Davis & Humphreys 2022). In the light of these data, the observed low genetic diversity represents a serious concern for the future of the population and the subspecies as a whole, and the intensive management should thus be continued. Further research, such as an assessment of whether the increased level of inbreeding negatively impacts fitness of the individuals, ought to be performed. Moreover, analysis of SNPs representative of the entire genome might provide a deeper understanding of the situation.

6.1.2. Nyala

The number of alleles and degree of heterozygosity found in nyala are similar to the values obtained by Grobler et al. (2005) from several wild populations of the species, indicating that the low genetic diversity in captivity might be a consequence of reduced variability in the wild, similarly to the case of mountain bongo. Moreover, the European EAZA nyala stock is based solely on 13 founders (Nolasco 2019), thus failing to reach the minimum of twenty unrelated wild animals commonly recommended for establishment of captive populations (Willis & Willis 2010; Ralls & Ballou 2013), and this extremely low number may have further reduced the initial genetic diversity of the captive stock. The current situation might equally be influenced by genetic drift, with the low founder genome equivalent, only reaching a value lower than 2 (Nolasco 2019), providing support for this assumption (Ballou et 2010).

On the other hand, the diminished size of the founder base is commonly linked to a high degree of inbreeding (Willoughby et al. 2015), which was not observed in nyala, thus implying that the current management of the captive population is efficient in preventing reproduction of related individuals. This might be associated with the high numbers of annually effectuated translocations among the different institutions, with transports of 30 animals, representing approximately 10% of the total EAZA population, reported in 2019 (Nolasco 2019).

The European EAZA nyala population might thus benefit from introduction of new individuals to increase the founder base and consequently improve genetic parameters.

However, institutions keeping animals from both of the clades revealed by mitochondrial DNA analyses were identified, which raises a concern if local adaptations are present, especially for future potential reintroductions of individuals into the wild (Cosson et al. 2007; Kleiman-Ruiz et al. 2019). Grobler et al. (2005) recognized the two groups as possible separate evolutionary significant units and advised against their mixing at least until the situation is investigated, nevertheless, no study on the subject has been published since, and further assessment of the situation is thus required.

6.1.3. Sitatunga

The high number of alleles and degree of heterozygosity seen in sitatunga might be related to the large size and wide distribution area of the wild population, as well as the relative numerousness of the founder animals, rather than the current management of the captive stock. It appears that there could be an issue with preventing reproduction among related animals, as indicated by the high value of inbreeding coefficient. Increased exchange of animals among institutions based on their relatedness is an important factor to be considered for effective management, nevertheless the limited availability of information on the ancestry of animals, stemming from the historically widespread herd management practices (Zwanzger 2003; unpublished) that have only recently been abandoned by numerous zoos (Species 360 2022), poses a challenge to the management.

However, the presence of the two clades identified by mitochondrial DNA analyses, could equally contribute to the elevated number of alleles and heterozygosity found in the captive stock if mixing of the lineages takes place. There is a high likelihood of such an occurrence as evidence of institutions keeping sitatungas from both of the genetically distinct groups has been found. This interbreeding of animals from different clades is concerning if local adaptations are present, which is highly plausible due to the largeness of the wild distribution area. Since no studies on the genetics of either captive or wild population of sitatunga have been published, more research would be needed to form definitive conclusions. However, the current European EAZA population could benefit from a more systematic management, especially aimed at reduction of inbreeding to increase future viability of the captive stock, and prevent severe loss of alleles and genetic diversity currently present within the species.

6.1.4. Lesser Kudu

Thorough management might serve as an explanation for the relatively large number of alleles and heterozygosity, as well as the low inbreeding coefficient found in lesser kudu, especially since the values of these parameters contrast with the small population size, barely exceeding 80 individuals, which commonly accelerates the loss of genetic diversity (Ballou et al. 2010; Willoughby et al. 2015). When compared to the other taxa analyzed in this thesis, the lesser kudu population bears the closest resemblance in terms of management intensity and availability of information to that of mountain bongo, nonetheless, it displays a much higher genetic variability despite being smaller and based on fewer founders. Consequently, other factors likely have an impact on the lesser kudu captive stock, such as a higher variability of the wild population, possibly linked to its larger size and more expansive distribution compared to the mountain bongo. The current management should be continued at a high intensity, especially since the number of wild lesser kudus is decreasing (IUCN SSC Antelope Specialist Group 2016c), and the genetic diversity of the captive population might thus be crucial for the future of the species.

6.1.5. Greater Kudu

Similarly to sitatunga, the high number of alleles and heterozygosity observed in greater kudu could be a consequence of a large genetic variability of the founders, associated with the numerousness and wide distribution range of the wild population. This assumption is further supported by the fact that most of the values found in greater kudu were comparable to those detected in sitatunga, despite the former having a significantly smaller captive population size. As a more rapid loss of genetic diversity would be anticipated in the captive stock comprising fewer individuals (Ballou et al. 2010; Willoughby et al. 2015), the discrepancy of this expectation and reality implies that the greater kudu founder population may have displayed greater variability than that of sitatunga.

Nevertheless, as two genetically distinct clades were distinguished in the species, and a case of an institution keeping animals from both of these groups was identified, the genetic diversity found in greater kudu could also be linked to the mixing of the two lineages, which may equally explain the observed negative value of inbreeding coefficient. This situation is concerning, as Jacobs et al. (2022) detected variation in adaptive loci, potentially linked to varying environmental conditions and divergent

morphology of greater kudus found in different portions of the wild distribution range, and Sakwa (2020) identified a genetically distinct population in South Africa, resulting from its prolonged separation from the remainder of the species, and advised against its interbreeding with animals from other areas of occurrence. The future captive population management should therefore be focused on prevention of reproduction of individuals from the different clades in order to retain their genetic uniqueness and reintroduction potential.

6.1.6. Common Eland

The situation of the European EAZA common eland stock closely resembles the one observed in the greater kudu, with the high degree of genetic diversity and low level of inbreeding potentially attributable to a combination of a large wild population size and distribution and interbreeding of the different lineages identified by mitochondrial DNA analyses. Further support to this assumption is provided by the presence of distinct genetic clusters within the population, revealed by the STRUCTURE analysis, as well as the statistically significant deviation from the Hardy-Weinberg equilibrium. An evidence of institutions keeping animals from genetically distinct groups was found, which is worrisome for the future of the species as Lorenzen et al. (2010) detected a significant divide in terms of genetic structuring and evolutionary pathways among the different lineages and recommended for this finding to be applied in further management of the species. However, decision-making in the case of common eland is hindered by the lack of available information on the ancestry of the animals, associated with the relatively recent transition from herd to individual management and the absence of a studbook. The future management should thus focus on prevention of interbreeding of animals from genetically distinct lineages, which could be aided by application of molecular methods.

6.2. Origin and Representativeness of the Populations

Determination of geographic origin of the maternal lineages constituting the European EAZA population using mitochondrial DNA was successful in all of the studied taxa apart from the lesser kudu, where it was encumbered by the lack of geographic structuring present within the species, consistently with the study of Bock et al. (2014), and the mountain bongo, where the precise identification of the place of origin was impossible due to the lack of variability present in the wild population. The analyzed samples were

found to reflect the majority of the wild distribution range in nyala, which may be linked to the small size of its area of occurrence. On the contrary, the populations of the remaining three species, sitatunga, greater kudu, and common eland, characterized by a wide distribution range, were observed to represent only a portion of the area of occurrence.

The situation in sitatunga reflects the available information on the origin of the founders, with the clustering of most samples with sequences from Congo, Cameroon, and eastern Gabon mirroring the fact that the vast majority of the founder animals was captured in Democratic Republic of Congo (Zwanzger 2003; unpublished), a country located within the same region. Likewise, the samples associating with the clade from western Gabon might potentially be a result of two of the founders originating from the neighboring Equatorial Guinea (Zwanzger 2003; unpublished). In addition to that, the EAZA captive population is sometimes referred to as the Western sitatunga (*Tragelaphus spekii gratus*) subspecies (Zwanzger 2003; unpublished), which predominantly occurs in the Congo Basin (Frost 2014). Although the results suggest that the core area of Western sitatunga distribution is represented in the European EAZA captive stock, individuals from some of the localities of the subspecies presence, such as Senegal and Gambia (Frost 2014), are missing.

All of the analyzed greater kudu samples were most closely associated with sequences from South Africa, Namibia, and Botswana, potentially indicating southern Africa as the sole region of the European EAZA population origin. Consequently, animals from eastern Africa and the westernmost area of the species distribution, comprising Chad, Central African Republic, Sudan, and South Sudan (Frost 2014), are missing in the European captive stock for it to achieve full representativeness of the wild distribution of the species. Similarly, the results suggest that although both the southern and northern extremes of the common eland wild distribution are embodied in the European EAZA population, despite the former being underrepresented, no animals from the central portion of the area of occurrence were identified.

Although studies on the geographic origin of zoo populations are scarce (Stanton et al. 2015), a varying degree of representativeness of the wild distribution range in the captive populations has been observed. Using analyses of mitochondrial DNA, large portions of the areas of natural occurrence were found to be embodied in the European captive

populations of the François' langur (*Trachypithecus francoisi*; Farré et al. 2022) and the binturong (*Arctictis binturong*; Cosson et al. 2007). On the contrary, an evaluation of multiple captive populations of ruffed lemurs (*Varecia* spp.) based on molecular data only identified the northern portion of the wild distribution range as the origin of the animals (Vega et al. 2023).

The analyses of mitochondrial DNA revealed the presence of two principal genetically distinct clades in four of the studied taxa, nyala, sitatunga, greater kudu, and common eland, and the analyzed samples were found to cluster with both groups in all of the species. In nyala and sitatunga, a rather even distribution of samples into the two clades was observed, implying a relatively high genetic representativeness of the captive populations. A similar situation was observed in several other animal taxa, such as the koala (*Phascolarctos cinereus*; Seddon et al. 2014) or western lowland gorilla (*Gorilla gorilla gorilla*; Soto-Calderón et al. 2015).

On the contrary, the distribution of the samples among the groups was skewed towards one of the clades in the greater kudu and common eland. In both of the species, a single animal associated with one of the groups, in contrast with a large number of samples clustering with the other, consistently with the findings of Ogden et al. (2018). Consequently, the haplotype diversity contained in the wild populations of these two species is far from being mirrored in the European EAZA captive stock in its entirety, and equalization of the representativeness of the distinct genetic groups should thus be focused on in future management. A discrepancy between the captive and wild population was equally observed in mountain bongo, where all of the analyzed samples were of a single haplotype, which is in agreements with the findings of O'Donoghue et al. (2017) and Sandri (2020). As the most common haplotype present in Kenya identified by Faria et al. (2011) was not detected in the European EAZA population, the genetic representativity of the captive stock is limited. Identical failure of the captive populations to genetically mirror the diversity found in the wild was reported in multiple species, such as okapi (*Okapia johnstoni*; Stanton et al. 2015), Madagascar big-headed turtle (*Erymnochelys madagascariensis*; White et al. 2022), and Matschie's tree kangaroo (*Dendrolagus matschiei;* McGreevy et al. 2008).

6.3. Limitations of the Thesis

One of the principal limitations of the genetic diversity analysis is the insufficient number of analyzed individuals, especially in the cases of mountain bongo and greater kudu, where only 10 and 17 animals were included in the dataset, respectively. These numbers fail to reach the minimum of 25-30 samples recommended for population studies using microsatellites by Hale et al. (2012) to reduce the error in determination of allele frequencies and heterozygosity estimates. Consequently, inclusion of more samples, representative of a larger proportion of the populations and institutions keeping them, would be needed to provide a more complex insight into the genetic parameters of the European EAZA captive populations.

In addition to that, two of the selected microsatellite loci failed to amplify in one species each, which is probably linked to the fact that the used markers are not specific to the studied taxa. Although microsatellites are capable of cross-specific amplification (Oliveira et al. 2006), the occurrence of mutations in different taxa might have a negative effect on the amplification process (Jarne & Lagoda 1996). As a result, only eight of the loci were amplified in all of the studied taxa, which decreased the comparative potential of the obtained results. Moreover, these loci are only representative of a small portion of the entire genome, and an increase in the number of used microsatellite loci or selection of more representative markers is thus advisable for future research.

The maternal lineages encompassed by the samples used for the mitochondrial DNA analyses reach high representativeness of the European EAZA populations in four of the taxa, mountain bongo, nyala, lesser kudu, and greater kudu, ranging from 65% to 91.5%. However, the extent to which the European EAZA captive stocks are mirrored by the samples in the remaining two species, sitatunga and common eland, is impossible to determine due to a large amount of missing data on ancestry, and the representativeness estimates, not exceeding 20%, are likely highly inaccurate and rather pessimistic.

Furthermore, the determination of the origin of individuals is hindered in taxa where only an insufficient number of sequences sourced from wild animals is available. This situation was observed in lesser kudu where sequences obtained from captive individuals had to be used as a reference, and as the determination of their region of origin was reliant on reconstruction of maternal ancestry from studbook data, it might be inaccurate and

consequently distort the results. Similarly, the lack of reference sequences in nyala and sitatunga did not allow for identification of a more precise origin of the individuals, and the analyzed samples were only successfully assigned to broader geographic regions.

7. Conclusions

Genetic parameters of European EAZA captive populations of the six selected spiral-horned antelope taxa were measured and evaluated in the context of the degree of management, population history, conservation status, and availability of information that might be useful in the decision-making process. Various factors and their combinations were proposed as potential explanations for the observed values. The low genetic diversity in mountain bongo and nyala might be linked to reduced genetic variability present in the wild populations, as well as the limited size of the captive stock in the former and low number of founders in the latter. On the contrary, the high values of the parameters of genetic diversity detected in the other four species are possibly linked to their comparatively larger wild distribution areas and population sizes, as well as thorough management in the lesser kudu. The effectiveness of management is possibly the primary factor affecting the level of inbreeding within the populations.

However, in sitatunga, greater kudu, and common eland, the high genetic diversity could equally be linked to mixing of the genetically distinct lineages identified within the species, which is worrisome if local adaptations are present. The fact that an evidence of institutions keeping animals from different clades was found provides opportunities for their interbreeding, therefore representing a concern for the future survival and reintroduction potential, and it should be addressed in their further management

Geographic origin of the majority of the analyzed captive individuals was successfully identified, albeit sometimes only as a broader area, rather than a specific country. The nyala population was found to be originating from two regions in southeastern Africa, the Congo Basin was identified as the source of the sitatunga population, southern Africa was determined as the area of origin of the greater kudu stock, and the common elands were found to be sourced from both southern and eastern Africa. The Kenyan origin of the mountain bongos was confirmed, nevertheless, association with specific localities was impossible due to the lack of control region variability present in the taxon. In the case of lesser kudu, determination of origin was unsuccessful due to the absence of clear genetic structure linked to geographic distribution of the species, as well as the insufficient number of available sequences sourced from wild animals.

The results showed the European EAZA populations to be geographically representative of the wild distribution range solely in nyala, a species found across an area limited in size, whereas they only mirrored a limited portion of the ranges of occurrence in the more widely-distributed species. Similarly, reduced genetic representativeness of the captive populations was observed in most of the studied taxa.

Even though this thesis does not encompass the whole European EAZA populations of the selected species and subspecies, it provides an insight into their genetic diversity and geographic origin, and it proposes potential implications of the detected values of genetic parameters for future management of the species. The obtained results will be used to create taxon-specific reports, which will be made available to the respective studbook keepers in order to be used in further management of the populations.

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Appendices

List of the Appendices:

Appendix 1: Overview of Institutions of Sample Collection

Table 15: Institutions of sample collection

NAME USED	OFFICIAL NAME	LOCATION
Africa Alive	ZSEA Ltd (Africa Alive!)	Kessingland (United Kingdom)
Boissière	Espace Zoologique la Boissière du Dore	La Boissière du Dore (France)
Dvůr Králové	Zoo Dvůr Králové, a.s.	Dvůr Králové nad Labem (Czechia)
Edinburgh	Edinburgh Zoo - Scottish National Zoo	Edinburgh (United Kingdom)
Exmoor	Exmoor Zoological Park	Barnstaple (United Kingdom)
Gaia Zoo	GaiaZoo, Kerkrade	Kerkrade (Netherlands)
Hannover	Zoo Hannover gGmbH	Hannover (Germany)
Hodenhagen	Serengeti-Park Hodenhagen	Hodenhagen (Germany)
Jihlava	Zoologická zahrada Jihlava	Jihlava (Czechia)
Knowsley	Knowsley Safari Park	Prescot (United Kingdom)
La Palmyre	Parc Zoologique de La Palmyre	Les Mathes (France)
Lány	University Farm Estate Lány	Lany (Czechia)
Marwell	Marwell Wildlife	Winchester (United Kingdom)
Munich	Münchner Tierpark Hellabrunn	Munich (Germany)
Peaugres	Safari de Peaugres	Peaugres (France)
Plaisance	African Safari	Plaisance du Touch (France)
Plzeň	Zoologická a botanická zahrada Plzeň	Plzeň (Czechia)
Sigean	Réserve Africaine de Sigean	Sigean (France)
Vienna	Schönbrunner Tiergarten GmbH	Vienna (Austria)

Appendix 2: Mitochondrial DNA Sequences

Mountain Bongo

Faria et al. 2011: EU040245.1; EU040246.1

Hassanin et al. 2012: JN632703.1

Nyala

Grobler et al. 2005: AY530163.1; AY530164.1; AY530165.1; AY530166.1; AY530167.1; AY530168.1; AY530169.1; AY530170.1; AY530171.1; AY530172.1; AY530173.1; AY530174.1; AY530175.1; AY530176.1; AY530177.1; AY530178.1; AY530179.1; AY530180.1; AY530181.1

Hassanin et al. 2012: JN632702.1

Sitatunga

Hassanin et al. 2012: EF536357.1

Hassanin et al. 2018: MH792168.1

Ntie et al. 2010: FJ823281.1; FJ823282.1; FJ823283.1; FJ823284.1; FJ823285.1; FJ823286.1

Lesser Kudu

Bock et al. 2014: HG931349.1; HG931351.1; HG931350.1; HG931352.1; HG931353.1; HG931354.1; HG931355.1; HG931356.1; HG931357.1; HG931358.1; HG931359.1; HG931360.1; HG931361.1; HG931362.1; HG931363.1; HG931364.1; HG931365.1; HG931366.1; HG931367.1; HG931368.1; HG931369.1; HG931371.1; HG931372.1; HG931373.1; HG931374.1; HG931375.1; HG931376.1 ; HG931377.1; HG931378.1; HG931379.1; HG931380.1; HG931381.1; HG931382.1; HG931383.1; HG931384.1; HG931385.1; HG931386.1; HG931387.1; HG931388.1; HG931389.1; HG931390.1; HG931391.1; HG931392.1; HG931393.1; HG931394.1; HG931395.1; HG931396.1; HG931397.1; HG931398.1

Hassanin et al. 2012: EF536356.1

Nersting & Arctander 2001: AF301711.1; AF301712.1

Zeyland et al. 2017 (direct submission to NCBI): KY628399.1

Greater Kudu

Hassanin et al. 2012: JN632708.1

Jacobs et al. 2022: OK642776.1; OK642775.1; OK642774.1; OK642773.1; OK642772.1; OK642771.1; OK642770.1; OK642769.1; OK642768.1; OK642767.1; OK642766.1; OK642765.1; OK642764.1; OK642763.1; OK642762.1; OK642761.1; OK642760.1; OK642759.1; OK642758.1; OK642757.1; OK642756.1; OK642755.1; OK642754.1; OK642753.1; OK642752.1; OK642751.1

Nersting & Arctander 2001: AF301621.1; AF301622.1; AF301623.1; AF301624.1; AF301625.1; AF301626.1; AF301627.1; AF301628.1; AF301629.1; AF301630.1; AF301631.1; AF301632.1; AF301633.1; AF301634.1; AF301635.1; AF301636.1; AF301637.1; AF301638.1; AF301639.1; AF301640.1; AF301641.1; AF301642.1; AF301643.1; AF301644.1; AF301645.1; AF301646.1; AF301647.1; AF301648.1; AF301649.1; AF301650.1; AF301651.1; AF301652.1; AF301653.1; AF301654.1; AF301655.1; AF301656.1; AF301657.1; AF301658.1; AF301659.1; AF301660.1; AF301661.1; AF301662.1; AF301663.1; AF301664.1; AF301665.1; AF301666.1; AF301667.1; AF301668.1; AF301669.1; AF301670.1; AF301671.1; AF301672.1; AF301673.1; AF301674.1; AF301675.1; AF301676.1; AF301677.1; AF301678.1; AF301679.1; AF301680.1; AF301681.1; AF301682.1; AF301683.1; AF301684.1; AF301685.1; AF301686.1; AF301687.1; AF301688.1; AF301689.1; AF301690.1; AF301691.1; AF301692.1; AF301693.1; AF301694.1; AF301695.1; AF301696.1; AF301697.1; AF301698.1; AF301699.1; AF301700.1; AF301701.1; AF301702.1; AF301703.1 ; AF301704.1; AF301705.1; AF301706.1; AF301707.1; AF301708.1; AF301709.1

Ogden et al. 2018: MG839216.1; MG839217.1

Common Eland

Hassanin et al. 2012: JN632704.1

Lorenzen et al. 2010: GQ388117.1; GQ388118.1; GQ388119.1; GQ388120.1; GQ388121.1; GQ388122.1; GQ388123.1; GQ388124.1; GQ388125.1; GQ388126.1; GQ388127.1; GQ388128.1; GQ388129.1; GQ388130.1; GQ388131.1; GQ388132.1; GQ388133.1; GQ388134.1; GQ388135.1; GQ388136.1; GQ388137.1; GQ388138.1; GQ388139.1; GQ388140.1; GQ388141.1; GQ388142.1 ; GQ388143.1; GQ388144.1; GQ388145.1; GQ388146.1; GQ388147.1; GQ388148.1; GQ388149.1; GQ388150.1; GQ388151.1; GQ388152.1; GQ388153.1; GQ388154.1; GQ388155.1; GQ388156.1; GQ388157.1; GQ388158.1; GQ388159.1; GQ388160.1; GQ388161.1; GQ388162.1; GQ388163.1; GQ388164.1; GQ388165.1; GQ388166.1; GQ388167.1; GQ388168.1; GQ388169.1; GQ388170.1; GQ388171.1; GQ388172.1; GQ388173.1; GQ388174.1; GQ388175.1; GQ388176.1; GQ388177.1; GQ388178.1; GQ388179.1; GQ388180.1; GQ388181.1; GQ388182.1; GQ388183.1; GQ388184.1; GQ388185.1; GQ388186.1; GQ388187.1; GQ388188.1; GQ388189.1; GQ388190.1; GQ388191.1; GQ388192.1; GQ388193.1; GQ388194.1; GQ388195.1; GQ388196.1; GQ388197.1; GQ388198.1; GQ388199.1; GQ388200.1; GQ388201.1; GQ388202.1; GQ388203.1; GQ388204.1; GQ388205.1; GQ388206.1; GQ388207.1; GQ388208.1; GQ388209.1; GQ388210.1; GQ388211.1; GQ388212.1; GQ388213.1; GQ388214.1; GQ388215.1; GQ388216.1; GQ388217.1; GQ388218.1; GQ388219.1 ; GQ388220.1; GQ388221.1; GQ388222.1; GQ388223.1; GQ388224.1; GQ388225.1; GQ388226.1; GQ388227.1; GQ388228.1; GQ388229.1; GQ388230.1; GQ388231.1; GQ388232.1; GQ388233.1; GQ388234.1; GQ388235.1; GQ388236.1; GQ388237.1; GQ388238.1

Ogden et al. 2018: MG839218.1; MG839219.1; MG839220.1

Appendix 3: Institutions of origin of microsatellite DNA samples

Table 16: Institutions of microsatellite DNA sample collection

MOUNTAIN BONGO	
INSTITUTION	**NUMBER OF SAMPLES**
Dvůr Králové	8
Gaia Zoo	1
Peaugres	1
NYALA	
INSTITUTION	**TOTAL NUMBER OF SAMPLES**
Dvůr Králové	27
Plzeň	10
Edinburgh	7
Vienna	6
Hannover	5
Jihlava	3
Marwell	3
Africa Alive	1
Munich	1
SITATUNGA	
INSTITUTION	**TOTAL NUMBER OF SAMPLES**
Dvůr Králové	19
Africa Alive	1
Exmoor	1
Hodenhagen	1
Knowsley	1
Plaisance	1
LESSER KUDU	
INSTITUTION	**TOTAL NUMBER OF SAMPLES**
Dvůr Králové	35
Hannover	2
Hodenhagen	1
GREATER KUDU	
INSTITUTION	**TOTAL NUMBER OF SAMPLES**
Dvůr Králové	15
Munich	2
COMMON ELAND	
INSTITUTION	**TOTAL NUMBER OF SAMPLES**
Dvůr Králové	16
Lány	9
Hannover	1
Munich	1

Appendix 4: Maternal Lineages in European EAZA Populations

Table 17: Maternal lineages of mountain bongo (Species360 2022)

MATERNAL LINEAGE	INDIVIDUALS	ZOOS
193	64	30
3 (Kenya)	63	31
165	18	14
322	9	3
2475	4	4
14 (Kenya, Aberdare NP)	3	2
737	2	1
1917	2	2
313	1	1
185 (Kenya, Naiwasha)	1	1

Table 18: Maternal lineages of nyala (Species360 2022)

MATERNAL LINEAGE	INDIVIDUALS	ZOOS
6 (South Africa)	68	21
344	22	8
31	17	5
3	15	5
1612	11	4
1497	10	6
530	10	4
32 (South Africa)	10	2
297	9	4
195	8	3
126	5	4
1957	5	1
423	4	3
670	1	1
unknown	16	8

Table 19: Maternal lineages of sitatunga (Species360 2022)

MATERNAL LINEAGE	INDIVIDUALS	ZOOS
6035	27	9
5222	23	5
5047	21	7
5913	20	6
5246	14	1
24975299	14	5
4220	13	2
4643	13	9
6616	13	4

Table 19: Maternal lineages of sitatunga (Species360 2022; continued)

MATERNAL LINEAGE	INDIVIDUALS	ZOOS
5376	9	4
6617	8	3
SCHMIDING / 930986	8	1
4971	7	1
5915	7	5
6141	7	2
5423	6	1
5432	5	2
5561	5	2
5630	5	1
7592	5	1
8194	5	3
8283	5	1
8409	5	1
8515	5	1
8749	5	1
4833	4	1
7731	4	2
7871	4	1
GVK14-00143	4	2
4840	3	3
7340	3	3
7380	3	1
7853	3	1
8167	3	2
8646	3	1
4909	2	2
5039	2	1
5562	2	1
6643	2	1
7063	2	1
7850	2	1
8182	2	1
8262	2	1
8369	2	1
8770	2	1
8870	2	2
7939	2	1
8821	2	2
THOIRY / M12004	2	1
4913	1	1
6139	1	1
6614	1	1
7012	1	1
7068	1	1

Table 19: Maternal lineages of sitatunga (Species360 2022; continued)

MATERNAL LINEAGE	INDIVIDUALS	ZOOS
7951	1	1
8050	1	1
8066	1	1
8086	1	1
8126	1	1
8248	1	1
8956	1	1
9219	1	1
RMP15-00278	1	1
9399	1	1
CHESTER / 788	1	1
NYIREGYHA / M00963	1	1
unknown	123	29

Table 20: Maternal lineages of lesser kudu (Species360 2022)

MATERNAL LINEAGE	INDIVIDUALS	ZOOS
125	29	4
103 (Kenya)	22	7
26 (Kenya)	15	8
106 (Kenya)	9	3
104 (Kenya)	6	3
7128	1	1

Table 21: Maternal lineages of greater kudu (Species360 2022)

MATERNAL LINEAGE	INDIVIDUALS	ZOOS
5 (Namibia)	61	19
483	41	17
500	22	6
615	16	8
831 (Namibia)	7	2
28 (unknown wild)	5	1
383 (unknown wild)	5	4
T173	5	3
1434	4	2
T295	3	1
T297	3	1
1504	2	1
213	1	1
T174	1	1
T234	1	1
unknown	9	4

Table 22: Maternal lineages of common eland (Species360 2022)

MATERNAL LINEAGE	INDIVIDUALS	ZOOS
MIG12-29254642	21	10
16862072	19	5
26709632	14	8
MIG12-29032131	14	1
27409667	13	4
MIG12-29591594	13	2
MIG12-29806448	13	2
WOBURN / ELA054	11	4
8664892	10	1
MIG12-29528527	10	2
MIG12-29027907	9	3
27655983	8	5
KWY16-00174	8	6
MIG12-29806449	8	1
WUPPERTAL / 77026	7	2
27689003	7	2
KWY16-00155	7	3
MIG12-29933742	7	4
QCP17-03849	7	1
10689407	6	2
KWY16-00164	6	4
MIG12-29749733	6	2
3098874	5	3
22739782	5	1
27406128	5	3
MIG12-28068609	5	2
MIG12-29806447	5	2
26180152	4	1
27689010	4	1
27946695	4	1
GWK15-01078	4	1
MIG12-28663247	4	1
LONGLEAT / EL808	4	1
TKC17-00066	4	1
20635495	3	1
21343161	3	2
27700317	3	1
27910630	3	2
25884018	3	1
27700315	3	1
MIG12-28121242	3	1
MIG12-28671859	3	1
MIG12-29421716	3	1
MIG12-30059476	3	1
25414051	2	1

Table 22: Maternal lineages of common eland; continued (Species360 2022)

MATERNAL LINEAGE	INDIVIDUALS	ZOOS
26977747	2	1
27530539	2	1
26180151	2	1
DGG11-00311	2	1
BNY13-00442	2	2
BNY13-00674	2	2
DGG11-00313	2	1
GWK15-01082	2	1
MIG12-28657862	2	1
PMQ12-00021	2	1
PMQ15-00586	2	1
RMP15-01050	2	2
RMP15-01074	2	2
RMP17-01698	2	2
RMP17-01699	2	1
WHJ12-00067	2	1
WHJ13-00164	2	2
WHJ13-00166	2	1
WHJ13-00173	2	1
WHJ16-00651	2	1
WHJ17-00774	2	1
WHJ17-00775	2	1
19988395	1	1
23330803	1	1
24115896	1	1
26018279	1	1
27399102	1	1
27700319	1	1
27662260	1	1
BRQ14-00026	1	1
DMP13-00114	1	1
FHM12-00256	1	1
MIG12-28121668	1	1
MIG12-28730570	1	1
MIG12-28730572	1	1
MIG12-29047081	1	1
MIG12-29421706	1	1
MIG12-29905047	1	1
PMQ13-00101	1	1
UNKNOWN / P20529	1	1
unknown	84	23