Flying High

Advancing genetic insights into Rüppell's griffon vultures

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MSc Thesis, Animal Breeding and Genomics

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Flying high: advancing genetic insight into Rüppell's griffon vultures
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- Abstract

The Rüppell's griffon vulture (*Gyps rueppelli*) faces a critical decline in numbers, receiving the critically endangered classification. One solution is the initiation of a reintroduction program utilizing the captive vulture population. To ensure successful reintroduction, it is imperative that the genetic makeup of the captive population closely resembles that of the wild population. However, currently, there is a lack of information concerning the genetic status of both the captive and wild populations. My research aims to enhance our genetic understanding of the Rüppell's griffon vulture. This is accomplished by analyzing the mtDNA cytb region to assess genetic diversity and population structure in captive and wild populations. Additionally, the research validates the maternal ancestor data recorded in the studbook and identifies vultures valuable for further genetic research. A total of 27 blood samples from the captive population, and 128 samples from wild vulture populations were employed in my research. Sequences were aligned and trimmed to 794 base pairs. The genetic analyses were performed using Mega X, Arlequin and RStudio to determine haplotypes, genetic diversity and population structure. Furthermore, the identification of genetically valuable vultures was carried out using PMxLite. The analysis revealed within the captive population a haplotype diversity of 0.3732 (± 0.0102) and a nucleotide diversity of 0.000488 (± 0.000527). Furthermore, three haplotypes were identified, with one being prevalent among the samples, resulting in a limited population structure. Comparisons with the wild Rüppell's griffon vulture population displayed similarities in haplotype networks and population structures. However, the wild population exhibited higher genetic diversity. Furthermore, the mtDNA data largely supported the recorded maternal ancestors in the studbook. Lastly, the in-depth investigation of the studbook data identified seven vultures as potential candidates for further genetic research. This research contributes to our genetic understanding of the Rüppell's griffon vultures. By expanding our knowledge, my study provides valuable insight that can be used to advance conservation objectives.

- **Acknowledgement**

Now that my MSc thesis is almost finalized, I would like to express my gratitude. First and foremost, I want to extent my sincere thanks to Diergaarde Blijdorp for their invaluable guidance throughout my research this past year, and especially to Maarten Vis and Jeroen Kappelhof. I always felt welcome at Diergaarde Blijdorp, where they provided me with a calm and supportive workspace. I am also appreciative of Diergaarde Blijdorp and Avifauna for allowing me to use the necessary samples required for my research. Special thanks go to Mirte Bosse for her continuous supervision and her assistance, including providing essential support and feedback when needed. Lastly, I want to express my gratitude to my parents and my boyfriend. They not only assisted me in completing my thesis but also ensured I took the necessary breaks when required. I'd also like to acknowledge my steadfast companions, Robert and Rogier, my support stuffed animal vultures, for providing the motivation I needed to preserve during this research journey.

- **Content**

1. Introduction

The population of Rüppell's griffon vulture (*Gyps rueppelli*), much like numerous other vulture populations, is rapidly declining. It has declined so much, 90% reduction observed within just three generations (Bird, 2020). As a consequence of this decrease, the International Union of Conservation of Nature (IUCN) has classified the vulture as critically endangered (Botha, et al., 2017; IUCN, 2021; Thiollay, 2006;). In Figure 1.1, we can observe the diminishing geographical range of the Rüppell's griffon vulture due to the extinction. Since 1908, two sub-species have been identified: the *Gyps rueppelli rueppelli* and the *Gyps rueppelli erlangeri.* However, their specific geographic origins remains undetermined (Verdoorn, 2004). The reduction in the population can primarily be attributed to two key factors: poisoning of their food source and the loss of their habitat (IUCN, 2021). The implications of this decline are concerning, given the indispensable role vultures play in ecosystems. Vultures serve as highly efficient scavengers, with their ability to remove carcasses, their primary food source. This contributes to the reduction of mammal scavenger interaction at carcass sites, reducing the potential spread of diseases (Davidović, et al., 2022; Ogada, et al., 2016).

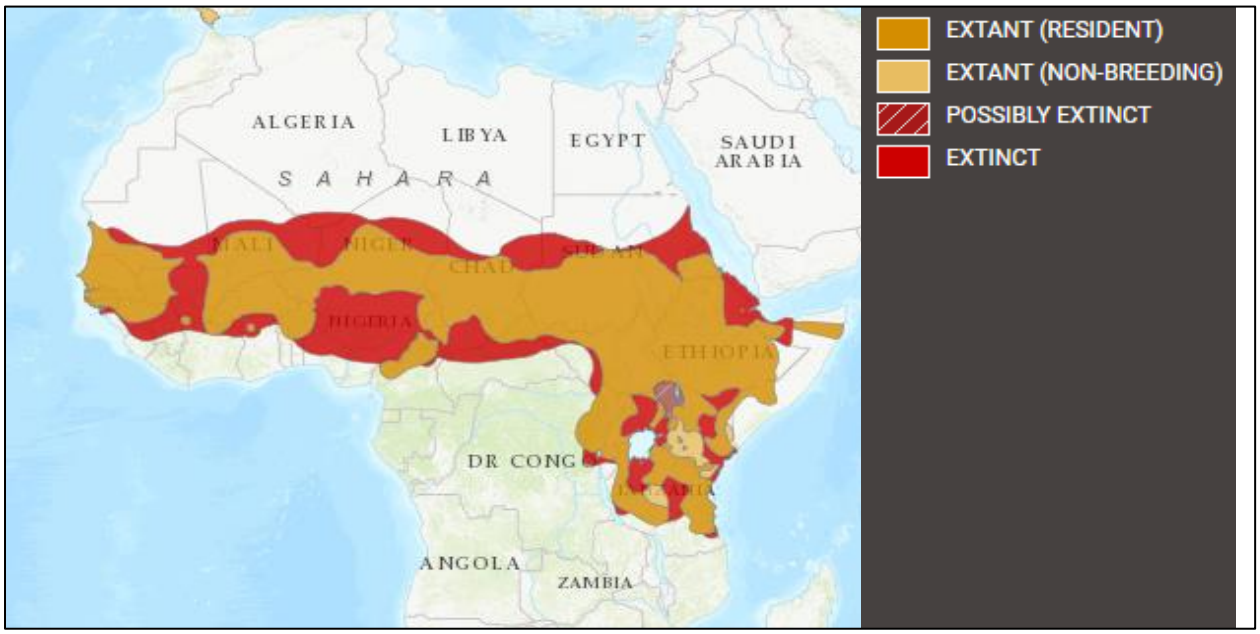

Figure 1.1: Visualization of the geographical range of the Rüppell's griffon vulture. This figure illustrates the present habitat distribution of the vulture, distinguishing between the current resident range and the region where the species has become extinct, based on data from IUCN (2021).

To counteract the declining Rüppell's griffon vulture population, various conservation measures have been instituted. Notably, in 2014, guidelines aimed at reducing the utilization of diclofenac, a veterinary medicine linked to the primary cause of vulture poisonings, were implemented (Ogada, et al., 2016). Additionally, the species is incorporated within the multi-species action plan focused on preserving African-Eurasian vulture species, initiated in 2017 (Botha, et al., 2017). Further efforts are being undertaken by organizations such as The Peregrine Fund to give education and create awareness regarding the significance of vultures on both local and global scales (IUCN, 2021; the Peregrine fund, n.d.). Despite these initiatives, the absence of a reintroduction program remains a notable gap in the conservation strategy. Nevertheless, the European Association of Zoos and Aquaria (EAZA)

has implemented an Ex situ breeding program, focusing on the captive reproduction of Rüppell's griffon vultures, with the ultimate goal of potentially releasing them back into the wild (Buij, et al., 2016). Diergaarde Blijdorp in Rotterdam serves as the studbook keeper for the captive Rüppell's griffon vulture population, and is responsible for all breeding management decisions (Pynnönen-Oudman, 2021). One essential aspect of the captive breeding program is to ensure that the captive population represents the wild population. A important concern in this regard is keeping the inbreeding factor as low as possible. This then maximizes the chances of successful reintroduction into the wild (Ford, 2002; Frankham, et al., 1986; Ivy & Lacy, 2010). Ensuring that the captive population accurately represents the genetic makeup of the wild population necessitates an in-depth look of their respective genetic diversity and population structures (Allendorf, et al., 2012; Forstmeier, et al., 2007). Population structure can be described as the diversity inherent in each population by scrutinizing allele or haplotype frequencies (Allendorf, et al., 2012). Meaning for the captive population to accurately replicate the wild population, it is imperative that the allele or haplotype frequencies in both populations align.

In order to conduct an in-depth analysis of population structure, the utilization of DNA or mitochondrial DNA (mtDNA), is imperative (Avise, et al., 1979; Barrett-Lennard, 2000). While Whole Genome Sequencing is the preferred method, utilizing mtDNA is an excellent starting point for genetic research. MtDNA is known for its stability, cost-effectiveness, and the wealth of existing data available from prior research studies (DeSalle, et al, 2017). Also, mtDNA possesses a unique characteristic, being exclusively inherited from one's maternal ancestor (Harrison, 1989). Presently, there exists accessible mtDNA data from wild Rüppell's griffon vultures in the National Center for Biotechnology Information (NCBI) databases. This dataset is specifically from the regions cytochrome b (cytb), recombination activating protein (RAG-1) and NADH dehydrogenase subunit 2 (ND2) (Arshad, et al., 2009; Johnson, et al., 2006; Lerner & Mindell, 2005). Cytb, a particularly variable and reliable region within the mtDNA, harbors three distinct haplotypes in the Rüppell's griffon vulture (Arshad, et al., 2009; Johnson, et al., 2006). Due to its variability, the cytb region is used in the majority of the DNA and mtDNA studies centered around the broader vulture family (Arshad, et al., 2009; Davidović, et al., 2022; Johnson, et al., 2006; Lerner & Mindell, 2005; Seibold & Helbig, 1995). Subsequently, the NCBI database contains substantial cytb data for various vulture species. Figure 1.2 outlines the geographical range and phylogenetic relationships of a select group of vulture species for which cytb data is available in the NCBI database. The inclusion of sequence data from related vulture species or those inhabiting the same geographical range holds significance as it facilitates the comparative analysis of their genetic structure, with the aim of determining whether uniform genetic patterns are evident across all vulture species (Arshad, et al., 2009; Johnson, et al., 2006).

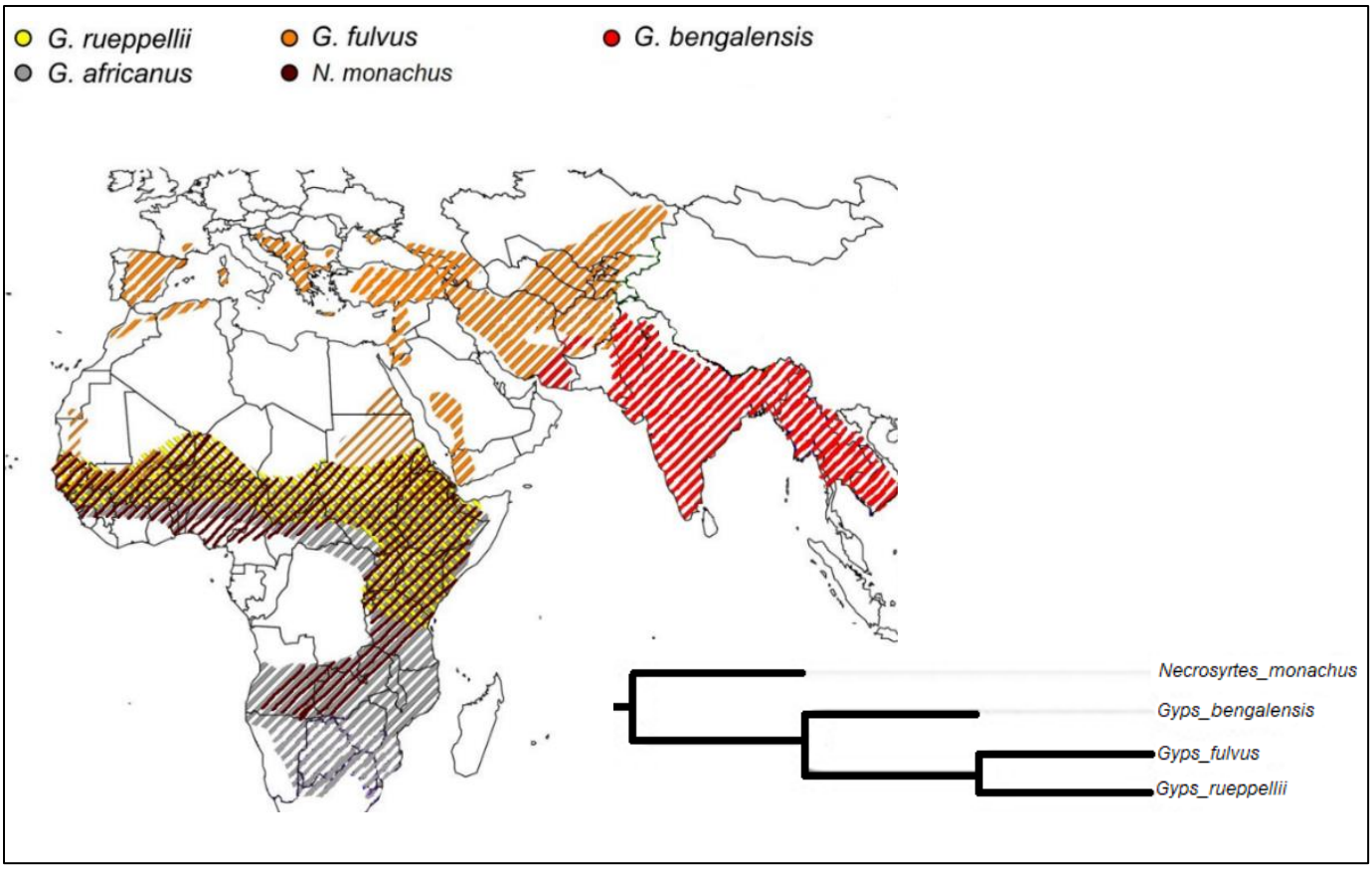

Figure 1.2: On the left, a comparative depiction of the geographical range of the Rüppell's griffon vulture in relation to four other vulture species, all of which possess recorded cytb data in the NCBI database (Johnson, et al., 2006). On the right, a phylogenetic tree encompassing four vulture species, including the Rüppell's griffon vulture, all of which have cytb data cataloged in the NCBI database (Mereu, et al., 2017).

Another integral part to a captive breeding program, is to have a accurately recorded studbook, which catalogues the correct lineage of individuals. This record then enables the calculation of the inbreeding coefficient and facilitates crucial management decisions, often accomplished through the use of specialized software, such as PMx (Jiménez-Mena, et al., 2016; Jones, et al., 2002). Furthermore, in combination with mtDNA analysis, it becomes feasible to establish the correct maternal ancestor, as disparities in mtDNA sequences indicate a lack of a mother-offspring relationship, while similarities provide support for such connections (Davidović, et al., 2022; Hutchison III, et al., 1974; White, et al., 2008).

Beyond the scope of the captive breeding program, there are ongoing endeavors to advance genetic research on the wild Rüppell's griffon vulture population. As illustrated in Figure 1.1, the species possesses an extensive geographic range spanning the sub-Sahara region of Africa. However, within this broad range, the numerous regional populations are dispersed, necessitating a precise delineation of their respective locations. This finer geographical understanding is imperative to advance genetic research and contribute to the conservation efforts aimed at safeguarding the wild Rüppell's griffon vulture population (Virani, et al., 2012). Current ongoing research aims to collect feathers in proximity to carcasses, a strategy designed to determine the population size, regional distribution, and the origins of vulture individuals. Feathers serve as a non-intrusive and convenient reservoir of genetic material, and research has shown you can collect enough DNA from a feather sample (Speller, et al., 2011). To facilitate this research, the development of a genetic screening tool is essential (Gautschi, et al., 2003; Rege & Lipner, 1992: Schwartz, et al., 2007). This tools holds the potential to not only define regional population locations but also to investigate the sub-species of the Rüppell's griffon vulture (Templeton, 1991). The genetic screening tool

could create genetic markers linked to different origin locations, thereby aiding in the identification of an individual vulture's place of origin. This information, indirectly, can improve conservation efforts by pinpointing regions requiring targeted conservation measures. The development of this genetic screening tool may necessitate the utilization of captive individuals, drawing upon the information within the studbook, such as place of origin, sex, or sub-species classification (Irizarry, et al., 2016; Templeton, 1991).

Additionally, the development of a reference genome, characterized by an assembled whole genome sequence from a representative individual of the species, holds substantial promise in advancing genetic research (Worley, et al., 2017). Reference genomes play a pivotal role in, for example, the identification of genetic adaptations and evolutionary modifications within a genome (Chung, et al., 2015; Worley, et al., 2017). Long-read sequencing technologies, such as the Oxford Nanopore Technology, are instrumental in constructing reference genomes (Lee, et al., 2019). The selection of an appropriate individual for this purpose is a critical consideration; it must not only be a faithful representation of the species but also provide high-quality DNA samples suitable for nanopore sequencing (Chen, et al., 2021). Presently, there is an absence of a reference genome for the Rüppell's griffon vulture. Nevertheless, ongoing genetic research endeavors are dedicated to the development of such a reference genome, as evidence by the 'Bird 10k' project (Zhang, 2015). Furthermore, Diergaarde Blijdorp has allocated substantial funding to the Wageningen Livestock Research laboratory for the execution of a nanopore sequence analysis, which will be conducted utilizing a sample from the captive Rüppell's griffon vulture population.

Genetic data holds paramount importance for potential reintroduction initiatives. However, a significant lack of such information, both concerning the captive and wild populations, poses a significant challenge. Additionally, a debate persists concerning the validity and distribution of sub-species, necessitating further genetic exploration. Consequently, my research project aims to enhance our genetic understanding of the Rüppell's griffon vulture. The objectives of this study is to determine the genetic diversity and population structures within the captive and wild populations of Rüppell's griffon cultures through comprehensive genetic analyses based on the mtDNA cytb region. Additionally, it aims to validate the correct maternal ancestor data within the captive studbook based again of the mtDNA cytb region. Furthermore, to identify individuals within the captive population that merit further genetic investigation, based on both mtDNA data and the information contained within the studbook. To address these objectives, the research will answer the following questions:

How much genetic diversity and population structure exist within the captive population?
    - How does the genetic diversity and population structure compare to that observed in the wild Rüppell's griffon vulture population?
    - How does the genetic diversity and population structure compare to vulture populations that are genetically close related or inhabiting the same geographical range?
- Does the analysis of mtDNA support the maternal ancestry data documented in the studbook?
- Which specific individuals within the captive population would be valuable for further genetic research, particularly in the development of a screening tool?

For the population structure I intend to integrate data from two closely related vulture species, the white-rumped vulture (*Gyps bengalensis*) and the Eurasian griffon vulture (*Gyps fulvus*). Furthermore, I will consider data from two vulture species that share the same geographical range, the hooded vulture (*Necrosyrtes monachus*) and the white-backed vulture (*Gyps africanus*). This approach is to compensate for potential gaps in data availability from wild Rüppell's griffon vultures. By employing genetic analyses of the cytb region within mtDNA of those vulture populations beside the wild population, I am able to determine the population structure and genetic diversity within the captive population and compare it to the wild situation. Furthermore, given the high variability of the cytb region, I think it will serve as an useful tool to evaluate the maternal ancestors documented in the studbook. Lastly, I think that the studbook currently contains the essential information that will facilitate the identification of individuals that may be valuable for further genetic research. At the end of my research I am able to answer all the research questions posed. Ultimately, the insights derived from these combined questions will play a significant role in enhancing the effectiveness of the captive breeding program and advancing the monitoring of the wild Rüppell's griffon vulture population.

2. Materials and Methods

2.1 Sampling

The sampling of the captive population of Rüppell's griffon vulture population took place on two distinct occasions, with samples gathered from Avifauna on October 31, 2022, and from Diergaarde Blijdorp on November 24, 2022. A total of 16 vultures in Avifauna and 8 vultures in Diergaarde Blijdorp provided blood samples, additionally besides blood, chest feathers were collected from the same 8 vultures in Diergaarde Blijdorp. The collection procedure adhered to the protocols specified by the EAZA biobank sampling guidelines (EAZA Biobank, n.d.). The samples were collected to facilitate research studies and also long-term preservation of these samples within the EAZA biobank. Subsequently, I was therefor also able to use these samples for my research. The collected blood samples were transferred into EDTA tubes, and stored at a temperature of -20 degrees Celsius within the Wageningen Livestock Research laboratory at Radix ABG.

2.2 Samples from previous research

Furthermore, my study made use of pre-existing blood samples from 4 Diergaarde Blijdorp vultures and feather samples from 6 Diergaarde Blijdorp vultures, which had been collected in 2021 and have been stored within the Wageningen Livestock Research laboratory at Radix ABG.

2.3 Data from online repositories

Additionally, I conducted a review of public available and published cytb mtDNA data, sourced from NCBI. This data encompassed wild individuals belonging to various vulture species, including *Gyps rueppelli* (Rüppell's griffon vulture), *Gyps fulvus* (Eurasian griffon vulture), *Gyps bengalensis* (White-rumped vulture), *Gyps africanus* (White-backed vulture) and *Necrosyrtes monachus* (Hooded vulture). A comprehensive list of these data sources is provided in Appendix 1.

2.4 DNA extraction

The extraction of mtDNA from blood samples was carried out using the Gentra Puregene Blood or Tissue kit, adhering to the protocol DNA purification from whole blood or bone marrow (Qiagen, 2007). The entire extraction process transpired within the controlled environment and using the equipment of the Wageningen Livestock Research laboratory at Radix ABG. No deviations from the established protocol were made. To establish the quality and purity of the mtDNA extracts, I used the Denovix DS-11 and Qubit 4 systems (table 2.1). For the extraction two key ratios were considered: the 260/280 and 260/230 values, which are considered acceptable when equal to or greater than 1.9, signifying high purity. Moreover, a minimum concentration value of 20 nanogram/µL was set as threshold for mtDNA sequencing (Desjardins & Conklin, 2010; Maghini, et al., 2021). Additionally, the quality of the mtDNA extracts was verified using Bio-Rad T100 Thermal Cycler and agarose gel electrophoresis (Appendix 2).

2.5 Sequencing

The cytb region of mtDNA was chosen for sequencing due to its high variability, as evidenced by the presence of three recorded haplotypes within the Rüppell's griffon vulture (Arshad, et al., 2009; Johnson, et al., 2006). Additionally, the availability of extensive cytb data on the NCBI database provided a valuable reference for sequence comparisons

(Arshad, et al., 2009; Davidović, et al., 2022; Johnson, et al., 2006; Lerner & Mindell, 2005; Seibold & Helbig, 1995). For the amplification of the cytb region, a set of primers was employed: Forward primer (OL23-007): 5' ACACCGCAGACACATCCTTA 3', and Reverse primer (OL23-010): 5' GAGGATGAGGAGATTGGCGA 3'. Prior to the polymerase chain reaction (PCR), a primer mix was prepared for individual primer. This involved combining 45 µl of a 5X dilution buffer, 30 µl of BD 3.1, 135 µl of MQ, and 60 µl of the respective primer. In each well of a 96-well plate, 1 µl of the PCR product was combined with 9 µl of the primer mix. The PCR process was carried out in the Bio-Rad T100 Thermal Cycler and consisted of an initial denaturation step at 96ºC for 5 min, followed by 30 amplification cycles: 96ºC for 30 seconds, 55ºC for 10 seconds, and 60ºC for 4 minutes. The reaction concluded with a hold step at 10ºC. The quality of the amplified products was confirmed through agarose gel electrophoresis (Appendix 3). Subsequently, the PCR products were purified using the Millipore PCR cleanup vacuum system (Multiscreen_PCR vacu 030). A detailed description of the complete purification procedure is provided in Appendix 4 for reference. Sequencing of the purified PCR products was executed using the ABI3730, a detailed description of the sequencing protocol in Appendix 5, which yielded both forward and reverse sequences. These sequences were combined into a single FASTA file for further analysis.

2.6 Data selection

Not all sample data was incorporated into my research for several deliberate reasons (Appendix 6). To maintain comparability and consistency in the analysis, I exclusively included blood samples in the dataset. Furthermore, to prevent redundancy, I excluded replicates of samples obtained from the same individual, thereby ensuring that genetic diversity and population structure comparisons with other vulture populations would remain unbiased. The choice of which sample to retain was based on assessment of quality, purity, and sequence length, with the overall highest-quality samples being selected. Notably samples BR1, BR2 and BR3, despite having lower concentration values than their corresponding replicates, were included in the study. Their lower concentration value was warranted by the fact that these samples had been pre-diluted prior to my research and their overall quality was higher than their replicates. Sample G2.2 was excluded because, while of good quality, it did not yield in a sequence of sufficient length to accommodate the location of SNPs and was thus deemed unsuitable for inclusion for my analysis.

2.7 Data analysis

All 155 cytb region sequences selected for analysis were aligned using MEGA 11, employing the Clustal W alignment option (Tamura, et al., 2021). Subsequently, they were trimmed to align with the length of the shortest available sequence, spanning positions 88 to 884. Furthermore, an exclusion of position 807 was undertaken, since not all samples had this base pair and it appeared to be a sequencing error, resulting in a total sequence length of 794 base pairs. I categorized the processed sequences into separate FASTA and MEG files based on the specific vulture populations to facilitate further analysis.

For the assessment of genetic diversity, I employed individual MEG files corresponding to different vulture populations. The calculations for the parameters: number of haplotypes and Tajima's D were performed in RStudio (Appendix 7) For the parameters: number of polymorphic sites, haplotype diversity, nucleotide diversity and haplotype frequency I utilized Arlequin version 3.5.2.2 (Excoffier, et al., 2005). Within Arlequin, I used the 'calculation

settings functions': 'Haplotype inference' and 'Molecular diversity indices' functions. These settings allowed for the determination of the specified parameters.

The analysis of population structure of each various vulture population involved the utilization of the individual FASTA files. Within RStudio, I employed these files to create and visualize the haplotype networks (Appendix 7). In addition, the MEG file containing the complete set of sequences for all vulture populations was utilized within MEGA 11 (Tamura, et al., 2021). These file was employed to construct a phylogenetic tree using the maximum likelihood method. The construction of the maximum likelihood tree was conducted within the 'Phylogeny' function using 'construct a maximum likelihood tree', with parameter 'Test of Phylogeny' which was configured to utilize the 'Bootstrap method' and the 'No. of Bootstrap Replications' set to a total of 10. The rest of the parameters was set according to the recommendations provided by the software.

Haplotypes were determined by using the MEG file from the captive Rüppell's griffon vulture. With the aid of the 'Sequence Data Explorer' function in MEGA 11 and then using 'highlight' the 'variable sites' (Tamura, et al., 2021). Subsequently, the exact SNPs and their locations were cataloged and combined resulting in the identification of haplotypes.

2.8 Studbook analysis

The studbook for the captive population of Rüppell's griffon vulture comprises records for 297 individual vultures. I accessed this studbook through a ZIMS file, which was then processed using the PMxLite software (Ballou, et al., 2022). Specifically, I navigated to the 'Genetics' tabs, utilized the 'Genetic Details' function, and exported the 'Individuals' information into an Excel file. This dataset provided the essential information required for my analysis of the captive population.

3. Results

For my research, I analyzed a total of 27 data samples from the cytb region of the captive Rüppell's griffon vulture population, along with 128 data samples from various wild vulture populations, including Rüppell's griffon vulture, White-rumped vulture, Eurasian griffon vulture, White-backed vulture and Hooded vulture. The wild vulture data were sourced from NCBI.

3.1 Genetic diversity and population structure

In the dataset obtained from the captive Rüppell's griffon vulture population, three distinct haplotypes were identified. The most prevalent haplotype, 12G 698T, was observed in 78% of all the samples. The second most frequent haplotype, 12 A 698T, was present in 18% of the samples. Lastly, haplotype 12G 698C was detected in 4% of all the samples. Notably, two of these haplotypes had not been previously documented in the NCBI database.

Table 3.1 reveals notable disparities in haplotype and nucleotide diversity between the wild Rüppell's griffon population exceed those of the captive population. Additionally, the sample size for the captive population considerably outweighs that of the wild population. Among the populations, the Eurasian griffon vulture was examined with the largest sample size, however, also with the lowest haplotype and nucleotide diversity. Furthermore, Tajima's D statistic, which measures departures from neutral evolution, yielded negative values for all populations, but statistical significance was only reached in the case of the Eurasian griffon vulture population. This suggests an excess of rare alleles in this particular population (Korneliussen, et al., 2013).

Table 3.1: a comparative analysis of standard parameters of genetic diversity based on the cytb region mtDNA sequence for all populations encompassing captive and wild Rüppell's griffon vultures, White-rumped vulture, Eurasian griffon vulture, White-backed vulture and Hooded vulture. N – number of samples, H – number of haplotypes, Nps – number of polymorphic sites, Hd – haplotype diversity, Nd – nucleotide diversity, Tajima's D – value of the Tajima's D statistical test with added p-value.

Population	N	H	Nps	Hd	Nd	Tajima's D (p-value)
Captive Rüppell's griffon vulture	27	3	2	0.3732 ± 0.0102	0.000488 ± 0.000527	-0.5360 (0.63)
Wild Rüppell's griffon vulture	4	2	1	0.5000 ± 0.0664	0.000630 ± 0.000775	-0.6124 (0.99)
White-rumped vulture	20	11	9	0.9474 ± 0.0004	0.003078 ± 0.001938	-0.1427 (0.92)
Eurasian griffon vulture	82	11	28	0.2927 ± 0.0044	0.001427 ± 0.001038	-2.4511 (0.00)
White-backed vulture	17	14	13	0.9779 ± 0.0005	0.003131 ± 0.001984	-1.3549 (0.18)
Hooded vulture	5	2	1	0.4000 ± 0.0570	0.000504 ± 0.000638	-0.8165 (0.60)

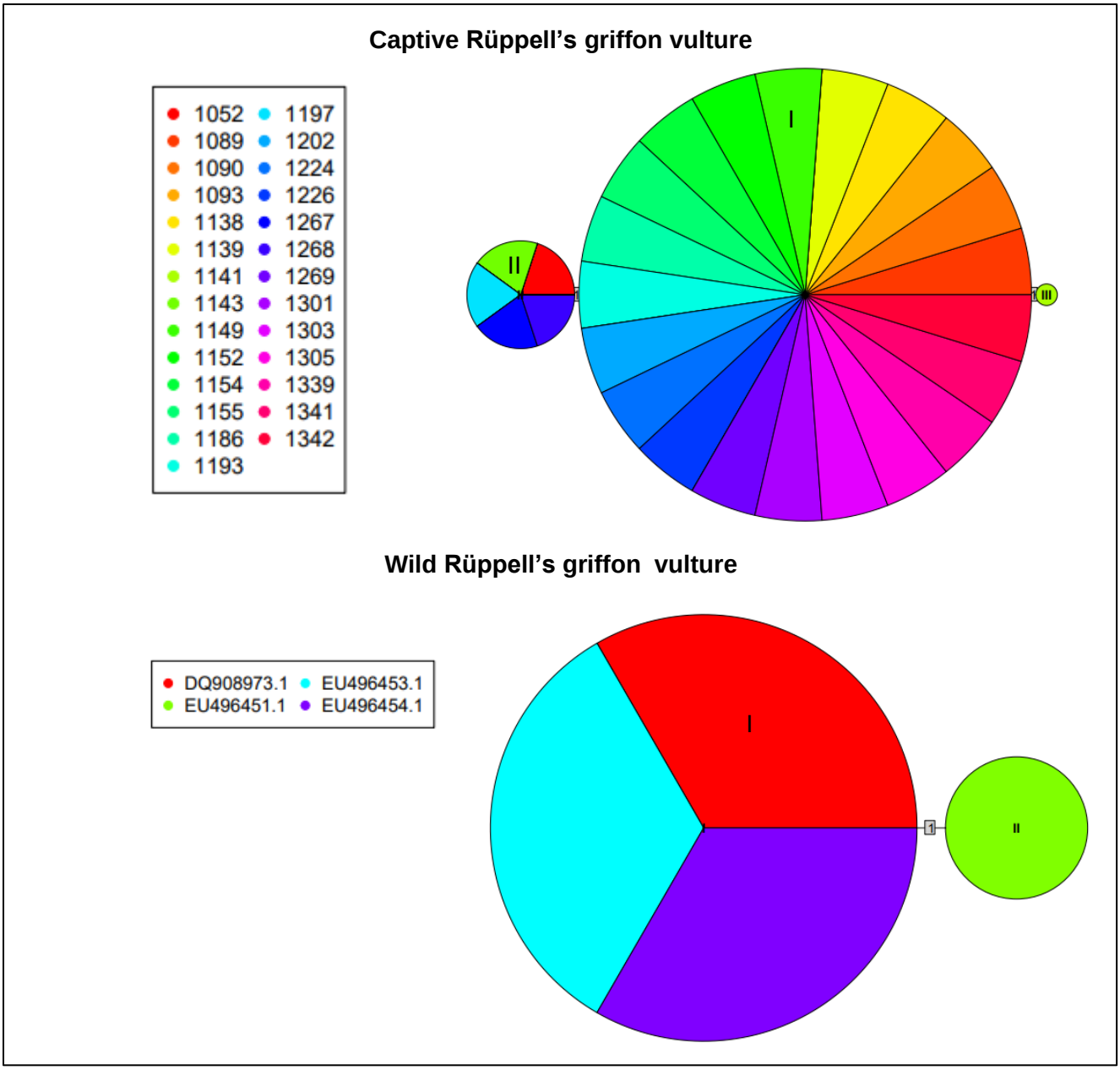

Figure 3.1: comparison of haplotype network between the captive and wild population of Rüppell's griffon vultures based on the cytb region of mtDNA.

Figure 3.1 illustrates that haplotype I in the captive population corresponds to haplotype I in the wild population. In contrast, neither haplotype II or III of the captive population corresponds to haplotype II of the wild population. Haplotype II and III are the haplotypes that are not documented in the NCBI database. Haplotype I represent vultures who have their residence in Avifauna and Diergaarde Blijdorp, while haplotype II and haplotype III only have vultures who have their residence in Avifauna. Both populations exhibit a high frequency of haplotype I, while the other haplotypes are significantly less common.

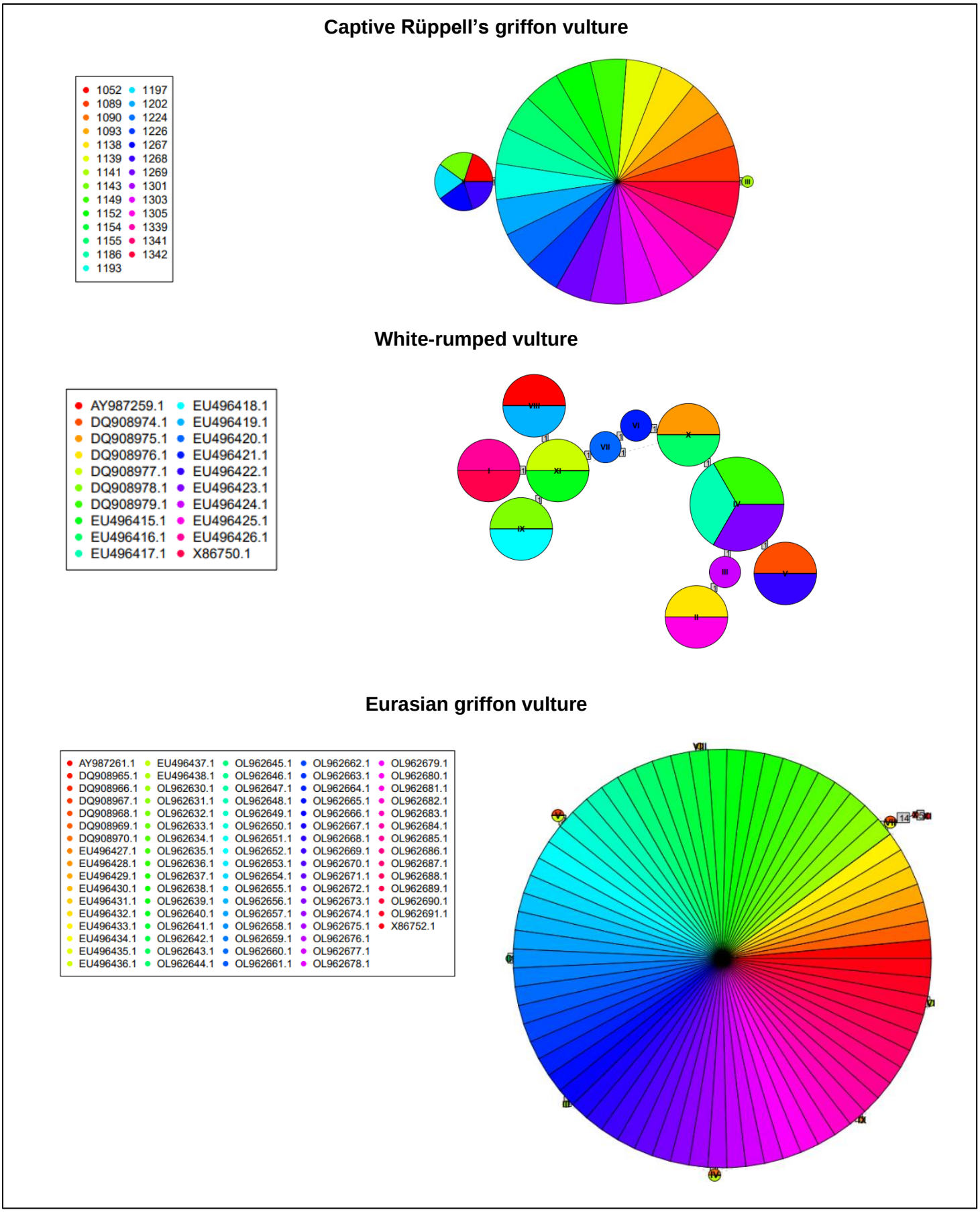

Figure 3.2: comparison of haplotype network between the captive and closely related vulture populations of the Rüppell's griffon vulture based on the cytb region of mtDNA.

In Figure 3.2 the Eurasian griffon vulture exhibits a pattern where one dominant haplotype prevails in the majority of the samples, while the remaining haplotypes are less frequent, a pattern akin to that observed in the captive Rüppell's griffon vulture population. However, in the case of the White-rumped vulture, the distribution of haplotypes is more uniform across the samples, with no clear dominance observed in one haplotype, unlike the pattern observed in the captive Rüppell's griffon vulture population.

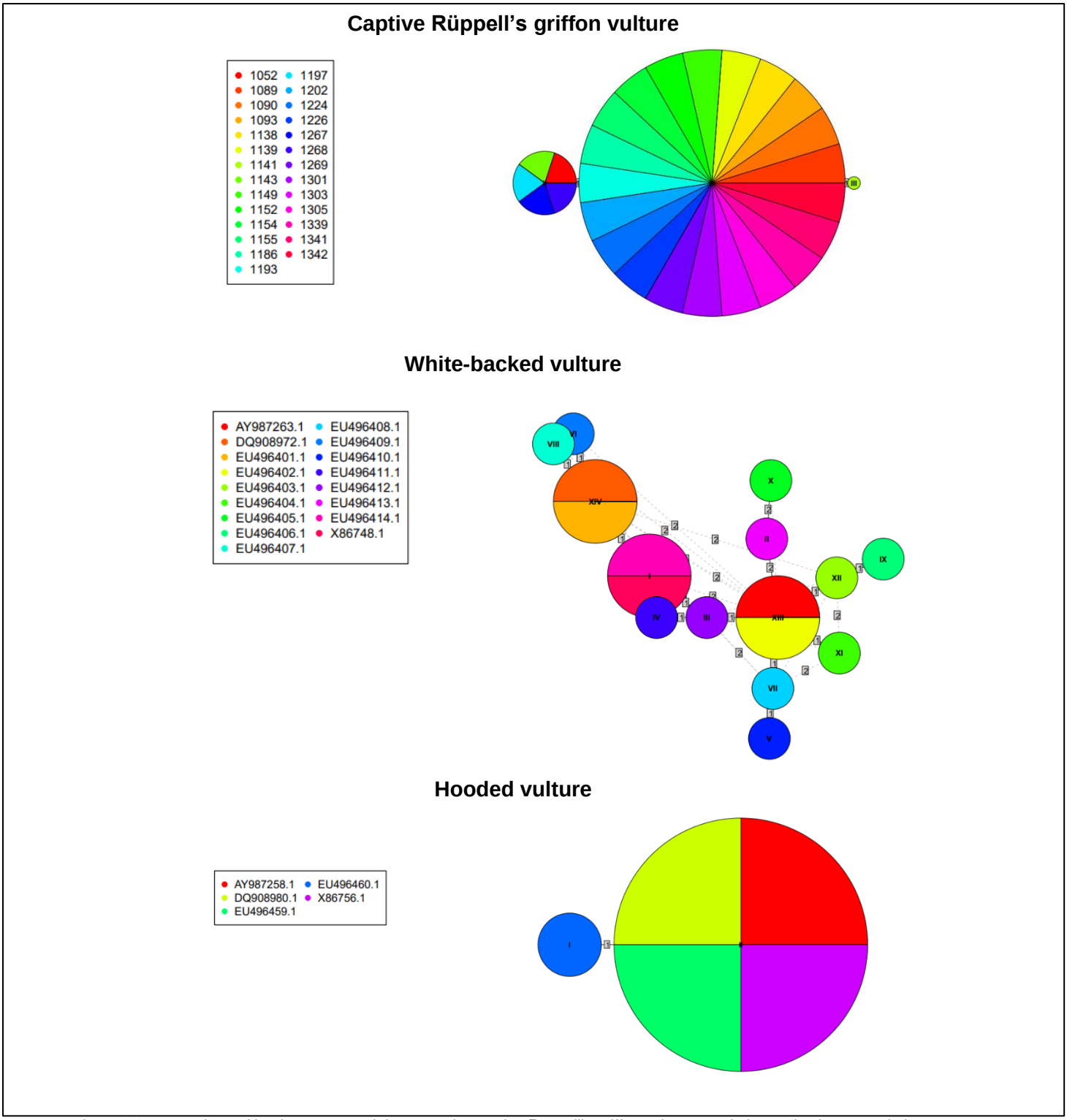

Figure 3.3: comparison of haplotype network between the captive Rüppell's griffon vulture population and vulture populations inhabiting the same geographical range based on the cytb region of mtDNA.

Similarly to the previous comparative analysis, when evaluating the Rüppell's griffon vulture population alongside vultures populations inhabiting the same geographical range in Figure 3.3, notable patterns emerge. The Hooded vulture population displays a haplotype network resembling that of the captive Rüppell's griffon vulture, characterized by the prevalence of a single major haplotype, with other haplotypes occurring less frequently. The White-backed vulture exhibits a distinct pattern in which a multitude of diverse haplotypes are distributed evenly within the haplotype network, differing form the captive Rüppell's griffon vulture population.

Additionally, in conjunction with the visual representation of haplotype networks, a phylogenetic tree containing all the different cytb haplotypes of all the vulture populations used in my study was constructed. This tree served to quantify the genetic distances within populations between their different haplotypes and allowed for a comparative analysis to determine if all vulture populations exhibited consistent genetic distances (Yan, L., n.d.). Bootstrap values were incorporated to provide statistical assessments of the reliability of various branches within the phylogenetic tree (Soltis & Soltis, 2003).

Figure 3.4 findings substantiate the patterns previously observed in the haplotype networks of the Rüppell's griffon vultures. The representation illustrates the dominance of a single major group in both the captive and wild populations, alongside isolated or smaller groups of samples. Notably, it visually confirms the shared present of the major haplotype I in both the captive and wild populations, while the smaller haplotypes are exclusive to either the captive or wild population. Genetic distances among these smaller haplotypes are consistent. The accompanying bootstrap values offer support for the reliability of these branches.

Figure 3.4 further highlights that no single haplotype is universally shared across all vulture populations, as each diverges into distinct groups representing their respective species. The Hooded vulture and the majority of the Eurasian griffon vulture populations exhibit a pattern akin to that observed in the Rüppell's griffon vulture, characterized by a prominent central cluster and several smaller groups dispersed along distinct branches. This pattern closely mirrors the visual representation of their haplotype networks. The genetic distance observed in the Hooded vulture population is comparable to those of the Rüppell's griffon vulture. The Eurasian griffon vulture has a higher genetic distance, however that is due to a unique divergence of two samples that are clustered with the White-rumped vulture. Conversely, the phylogenetic trees for the White-rumped and White-backed vultures, unveil a distinctive configuration, diverging from the pattern observed in Rüppell's griffon vulture. These populations lack a single dominant group, instead featuring multiple groups of similar sizes branching out. This pattern aligns with the visual representation of their haplotype networks. Notably, both these populations exhibit higher genetic distances when compared to the Rüppell's griffon vulture, with the White-rumped vulture and the Eurasian griffon vulture experiencing the highest genetic distance among all the vulture populations. The branches are supported by bootstrap values. Additionally in Appendix 7.8 all phylogenetic trees for the individual vulture populations are presented.

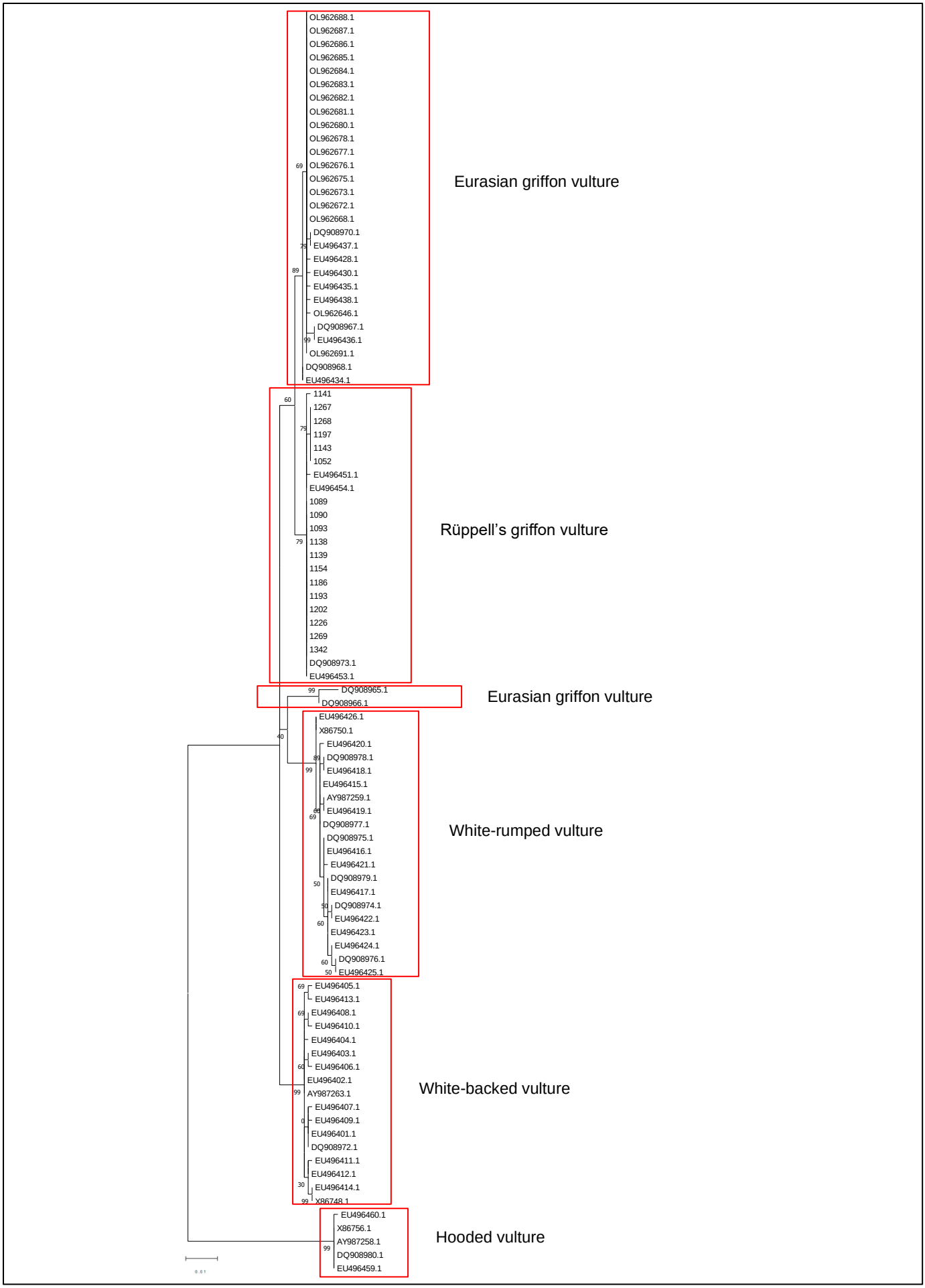

Figure 3.4: Phylogenetic tree depicting the genetic relationships among all haplotypes of the vulture populations included in this study. The tree is constructed based on the variability of the cytb region sequences using the ML method. Genetic distances are included, and bootstrap branch support values are assigned to each branch. The samples starting with a letter indicate wild vultures, and starting with a number indicate the captive Rüppell's griffon vultures.

3.2 Maternal ancestors

These result focus on if the mtDNA data supports the maternal ancestor information recorded in the studbook. In Table 3.2, data regarding mtDNA haplotypes is presented, encompassing 15 vulture samples. This table consist of a comparison between each vulture's individual cytb haplotype and the maternal ancestor's haplotype, extracted from the records in the studbook. However, it is important to note that not all 27 samples were included in this table. The exclusion of 12 male vultures was necessitated by the absence of available mtDNA cytb sequences for their respective maternal ancestors since they originated from the wild. Appendix 7.9 lists the cytb haplotypes of all 27 vulture samples, if necessary for further information. Conversely, three female vultures with wild maternal ancestors were included in the table, as their individual cytb haplotype data may provide insight into their potential role as maternal ancestor for the other captive vultures. Additionally, one vulture's maternal ancestor remained undetermined, however, the cytb haplotype data may facilitate the determination of the maternal ancestor. Lastly, there were two cases where two possible maternal ancestor were recorded in the studbook. The cytb haplotype data can be valuable in determining the correct maternal ancestor.

Table 3.2: mtDNA haplotypes based on the cytb region of the captive vultures from Diergaarde Blijdorp and Avifauna. The maternal ancestor documented in the studbook are included for haplotype comparison.

Vultures ID	Sex	Maternal ancestor according to the studbook	Location	Cytb haplotype	Maternal ancestor according to the studbook cytb haplotype
1052	Female	Unknown	Avifauna	12A 698T	Unknown
1093	Female	Unknown	Blijdorp	12G 698T	Unknown
1186	Female	Unknown	Avifauna	12G 698T	Unknown
1193	Female	1093	Blijdorp	12G 698T	12G 698T
1197	Female	Undertermined	Avifauna	12A 698T	Unknown
1202	Female	1093	Blijdorp	12G 698T	12G 698T
1267	Female	1052/1197	Avifauna	12A 698T	12A 698T/12A 698T
1268	Female	1052/1197	Avifauna	12A 698T	12A 698T/12A 698T
1269	Female	1185	Avifauna	12G 698T	Unknown
1301	Male	1197	Blijdorp	12G 698T	12A 698T
1303	Male	1093	Blijdorp	12G 698T	12G 698T
1305	Male	1193	Blijdorp	12G 698T	12G 698T
1339	Female	1193	Blijdorp	12G 698T	12G 698T
1341	Female	1193	Avifauna	12G 698T	12G 698T
1342	Female	1093	Avifauna	12G 698T	12G 698T

In Table 3.2, the haplotypes of several vultures, namely 1193, 1202, 1303, 1305, 1339, 1341 and 1342, were found to be in agreement with the corresponding maternal ancestors recorded in the studbook. However, vulture 1301 exhibited a discrepancy in the first SNP when compared to the studbook recorded maternal ancestor. For vulture 1197, whose maternal ancestor remains to be undetermined. The birth records indicate its origin in Avifauna, and based on this information and the cytb haplotype data, the potential maternal ancestors are vultures 1052, 1267, or 1268.

Lastly, the cytb haplotype data for vultures 1267 and 1268 does not provide conclusive evidence to differentiate between their two possible maternal ancestor candidates, as there are no differences in the haplotypes.

3.3 Vultures valuable for further genetic research

The selection of vultures valuable for further genetic research involved a comprehensive assessment of the captive Rüppell's griffon vulture studbook. The complete studbook encompasses 297 individual vultures. Among these, 159 are currently alive and available for sample collection, with a unique case of vulture 1304, which passed away in 2022, but a blood sample was preserved and thus qualifies for inclusion in further genetic research. For the remaining 165 vultures that are no longer alive, the availability of blood or feather samples is unknown, precluding their consideration for genetic research. This filtering process ultimately resulted in 160 vultures for further categorization. The primary objective of the ongoing genetic research is to create a screening tool for identifying the origin of individuals, necessitating the establishment of genetic markers specific to different origin locations, particularly focusing on the western and eastern edges of the Rüppell's griffon vulture geographical range. Consequently, the focus was placed on vultures born in the wild and possessing information about their birth location. This selection criterion led to the identification of four vultures, as detailed in Table 3.3. Notably, three of these vulture originated in West Africa, while one was from East Africa, thereby offering a valuable means of enhancing the representativeness of the screening tool across the western and eastern edges of the Rüppell's griffon vulture geographical range. One of these samples is already in possession of the Wageningen Livestock Research laboratory at Radix ABG, while the remaining three await collection. The next step involves contacting the respective zoos housing these vultures to request the acquisition of blood and/or feather samples for further research.

Another valuable application of the screening tool lies in the establishment of genetic markers to distinguish between the two sub-species of Rüppell's griffon vultures. There remains uncertainty regarding the precise origin location of these sub-species and whether they too exhibit a division between the western and eastern edges, necessitating the development of a distinct screening tool tailored to sub-species differentiation (Verdoorn, 2004). Within the studbook, two vultures are registered as the sub-species *Gyps rueppelli erlangeri* (Table 3.3), comprising of a mother and her offspring. The remaining vultures are categorized as *Gyps rueppelli rueppelli*. For further research endeavors, the genetic material from the mother or son vulture, could be utilized to create a genetic marker for the *Gyps rueppelli erlangeri*. Likewise, a sample from one of the other captive vultures, such as those utilized for the origin screening tool, could serve as a reference for the *Gyps rueppelli rueppelli*. These could both serve as a reference for validating and identifying sub-species in the wild.

Table 3.3: vulture valuable for further genetic research, including their place or origin, sub-species registration, current zoo location, and availability of blood/feather samples in the EAZA biobank or Wageningen Livestock Research laboratory at Radix ABG

Vulture ID	Place of origin	Sub-species	Zoo Location	Sample collected?
1051	Côte d'Ivoire (West Africa)	Gyps rueppelli rueppelli	Coulange	No
1052	Côte d'Ivoire (West Africa)	Gyps rueppelli rueppelli	Alphen	Yes
1103	United republic of Tanzania (East Africa)	Gyps rueppelli rueppelli	Hilvarenb	No
1118	Guinea (West Africa)	Gyps rueppelli rueppelli	Lesna	No
1106	Undetermined	Gyps rueppelli erlangeri	Thoiry	Yes
1265	Quintasi (captive born)	Gyps rueppelli erlangeri	Thoiry	No

Finally, I conducted the selection process for the vulture sample designated for the nanopore sequencing conducted at the Wageningen Livestock Research laboratory at Radix ABG. The chosen individual had to be selected from the samples available in the laboratory (documented in Appendix 6). My objective was to select an individual that would serve as a good representation of the entire Rüppell's griffon vulture population. I decided that it was there for best to look at the individuals born in the wild. Considering, that than their genetic data would be uncompromised by breeding management decisions. This choice aimed to ensure the genetic information derived from this individual would be more representative of the natural state of the population. I also preferred a female individual so that there is information of both of the sex chromosomes (Ghorpade, et al., 2012). Lastly, to check for the DNA quality, I had a look at the Qubit and Denovix results and looked at the agarose gel pictures to take the best quality sample. Ultimately leading to the selection of vulture 1093 as the prime candidate for analysis using the Oxford Nanopore sequencer. Sequencing this sample resulted in high-quality nanopore reads with sample name Vulture 409995, Total_Gb of 48.31, Avg_N50 of 35.61 and Genome coverage of 38.

4. Discussion

In this section of my research, I will delve into the discussion of the obtained results. However, first it is imperative to examine the method employed to acquire them. The choice to analyze the cytb region of mtDNA was because of its high variability and the wealth of available data in this region on NCBI (Arshad, et al., 2009; Johnson, et al., 2006; Lerner & Mindell, 2005). While alternative mtDNA regions, such as the D-loop or control region, exhibit even higher variability compared to the cytb region, they come with inherent drawbacks in terms of reliability (Arshad, et al., 2009; Nicholls & Minczuk, 2014). Thus, for my study, the cytb region was selected as the preferred genetic marker. Nonetheless, for future genetic studies, it is advisable to include not only the cytb region but also these additional regions to gain deeper insights into the genetic information of the Rüppell's griffon vulture.

Another methodological consideration was the decision to trim the sequences, ensuring uniform lengths across all samples. This facilitates meaningful comparisons of genetic diversity and population structure among the vulture populations (Guo, et al., 2021). However, a longer sequence fragment could potentially yield different outcomes. For instance, when examining previously recorded Rüppell's griffon vulture haplotypes, it was not feasible to identify certain haplotypes due to the sequence length limitation (Arshad, et al., 2009; Johnson, et al., 2006). The restriction in sequence length was primarily due to challenges in selecting ideal primers for sequencing the complete cytb region. Thus, for future research, the identification of more suitable primers to enable the sequencing of the entire cytb region is a valuable addition.

4.1 Genetic diversity and population structure

For this part of my research, the primary objective was to assess the genetic diversity and population structure of the captive Rüppell's griffon vulture population and compare it to wild populations. As previously emphasized, it is important to ensure that the captive population represents the wild population to maximize the change of successful reintroduction (Ford, 2002; Frankham, et al., 1986; Ivy & Lacy, 2010). Therefore, let us examine whether the captive population fulfills this requirement.

Upon comparing the captive population to the wild Rüppell's griffon vulture population, it becomes apparent that they share visual similarities in both the haplotype network and phylogenetic analysis. In both cases, a predominant haplotype or branch prevails, with the remainder partitioned into smaller, distinct ones. Additionally, the number of haplotypes and polymorphic sites in both population proves to be comparable. However, a crucial point of divergence emerges when considering parameters indicative of genetic diversity, the haplotype and nucleotide diversity. In this regard, the captive population exhibits a lower genetic diversity compared to the wild population. This discrepancy implies that, genetically, the captive population does not mirror its wild counterpart. Consequently, this disparity warrant consideration in captive breeding management decisions. One plausible explanation for this discrepancy could be unequal sampling. The samples of the wild Rüppell's griffon vulture population were collected from two distinct locations in Africa, specifically the western and eastern edges (Arshad, et al., 2009). In contrast, the precise origins of my samples

remain undisclosed, and it must be acknowledged that some stem from the same familial lineage. It is possible that all my samples are representative of a single origin location, thereby impacting the observed genetic diversity. An additional piece of evidence to support the notion that my samples originate from a different origin location than the location used for the collection of the wild samples is the discovery of two new haplotypes not documented in the NCBI database. Given that certain vultures in my dataset originated from the wild, it is highly probably that these newly identified haplotypes also exist in the wild population, yet remain uncollected. Furthermore, it is noteworthy that my samples solely originate from Diergaarde Blijdorp and Avifauna, and other zoos could possible house vulture families that potentially harbor distinct haplotypes. This is evident in the exclusive presence of haplotype II and haplotype III in the samples collected form Avifauna. Hence, an expansion of sample collection from various zoos is likely to offer a more comprehensive result on the genetic diversity within the captive Rüppell's griffon vulture population. If, following this extension, the genetic diversity of the captive population still lags behind that of the wild population, a viable solution could involve prioritizing vultures bearing the rarer haplotypes for breeding purposes to increase genetic diversity (Amos & Harwood, 1998; Harpur, et al., 2012).

Another significant facet of my research was the comparative analysis of various vulture populations with respect to the Rüppell's griffon vulture population. These vulture populations exhibit difference not only in geographic range but also in their phylogenetic relatedness to the Rüppell's griffon vulture. Hence, my research was focused on how these factors would impact their genetic diversity and population structure. Upon analyzing genetic diversity, it was evident that the Eurasian griffon vulture exhibited the lowest genetic diversity parameters among the vulture populations. Notably, when compared to the other vulture populations, the Eurasian griffon vulture faced a lesser threat of extinction, bearing the 'Least Concern' classification according to the IUCN, in stark contrast to the 'Critically Endangered' classification of all other vulture populations (BirdLife International, 2021a; BirdLife International, 2021b; BirdLife International, 2021c; BirdLife International, 2021d) . The remaining vulture populations, in comparison to the captive Rüppell's griffon vulture population, displayed higher genetic diversity. The low genetic diversity observed in the Eurasian griffon vulture population, once more, may be ascribed to unequal sampling. The predominance of Eurasian griffon vulture samples in my dataset emanated from a singular study conducted in Serbia, indicating a concentration of samples from that specific location (Davidović, et al., 2022). Such unequal sampling may, in part, account for the observed lower genetic diversity in the Eurasian griffon vulture in contrast to other vulture populations. The higher genetic diversities observed in other vulture populations underscore the significance of conserving the relatively low genetic diversity in the captive Rüppell's griffon vulture population when formulating breeding management decisions.

When examining the comparison of haplotype networks between the captive Rüppell's griffon vulture and other vulture populations, it became evident that the Eurasian griffon vulture and Hooded vulture, much like the Rüppell's griffon vulture, displayed a predominant haplotype with smaller ones. In contrast, the White-rumped and White-backed vulture haplotype networks deviated from the Rüppell's griffon vulture, presenting a more dispersed network without a distinct major haplotype. The underlying reasons for these disparities remain unknown. This is because even among closely related vulture species of the Rüppell's griffon vulture such as the Eurasian griffon vulture and White-rumped vulture, distinct haplotype networks were observed. Similarly, vulture populations inhabiting the

same geographical range, like the Hooded vulture and White-backed vulture, also exhibited differing haplotype networks. However, it is still something to consider that the captive Rüppell's griffon vulture population may not adequately represent all wild vulture populations when looking at the haplotype network. Additionally, the analysis of the phylogenetic tree of all the vulture populations yielded valuable insights. The majority of vulture populations, including the White-backed vulture, White-rumped vulture, and the Eurasian griffon vulture, displayed a higher genetic distance compared to the Rüppell's griffon vulture. This suggests that more SNPs contribute to defining the various haplotypes in these populations compared to the Rüppell's griffon vulture, which is defined by just two SNPs (Leaché, et al., 2015). This implies that the Rüppell's griffon vulture exhibits relatively lower genetic diversity when contrasted with other vulture populations.

4.2 Maternal ancestor

Reflecting back on the research questions, the primary aim was to validate the recorded maternal ancestor data within the captive Rüppell's griffon vulture studbook. As previously emphasized, a correctly recorded studbook plays an essential role in upholding the genetic quality and diversity of a population, which, in turn, indirectly supports the conservation of a species (Jiménez-Mena, et al., 2016; Jones, et al., 2002.

In the case of the captive Rüppell's griffon vultures, a substantial number of individual vultures exhibited cytb haplotype identical with those of their suggested maternal ancestor. This conformity suggests that these vultures have indeed inherited their mtDNA haplotypes from their maternal ancestor, aligning with the expectations surrounding mtDNA inheritance (Davidović, et al., 2022; Hutchison III, et al., 1974; White, et al., 2008). However, since there was one major haplotype present in the captive Rüppell's griffon vulture population it is also possible that they coincidently have the same haplotype Thus, it is not yet possible to say with certainty that the mtDNA supports their suggested maternal ancestors. Also, it is worth noting an exception in the case of vulture 1301, where the observed haplotype differed from that of the suggested maternal ancestor. Since mtDNA is exclusively inheritable from the maternal lineage, such discrepancies are biologically implausible (Davidović, et al., 2022; Hutchison III, et al., 1974; White, et al., 2008). This raises the possibility of an error in the identification of the maternal lineage for vulture 1301. In the studbook, vulture 1197 was designated as the maternal ancestor of vulture 1301, but the basis for this determination remains unclear. It is possible that a procedural error during the registration of the maternal ancestor may have occurred. Additionally, it is essential to acknowledge that the sequencing of mtDNA is not immune to errors, and a misplaced nucleotide base at position 12 during the sequencing process could theoretically account for the observed discrepancy (Bandelt, et al., 2001). In that scenario, vulture 1301 might indeed share the same haplotype as the suggested maternal ancestor, thus validating the studbook's records.

My research exclusively examined the cytb region of the mtDNA. Expanding the analysis to include multiple regions of mtDNA in the future would be a valuable step. This broader scope could potentially reduce the error rate and provide more robust evidence for the determination of the maternal ancestors of the vultures (Davidović, et al., 2022). As illustrated by the cases of vultures 1267 and 1268, who each had two suggested maternal ancestors, the cytb haplotype alone did not exclude either possibility. Incorporating additional mtDNA regions would likely offer a more comprehensive dataset for maternal

lineage identification (Davidović, et al., 2022; Hutchison III, et al., 1974; White, et al., 2008). A similar approach could be applied to vulture 1197, with the prospect that more mtDNA data may facilitate the correct determination of the maternal ancestor. During my exploration of NCBI, I encountered mtDNA data of the regions of RAG-1 and ND2 with different haplotypes, indicating variation in those regions (Arshad, et al., 2009; Johnson, et al., 2006; Lerner & Mindell, 2005). Additionally, other studies on maternal ancestry determination have incorporated the highly variable neutral D-loop region in mtDNA (Davidović, et al., 2022). Therefore, for future research, the inclusion of one or more of these regions, alongside the cytb region, could provide a more comprehensive and insightful approach to identifying the maternal ancestors of the captive Rüppell's griffon vultures.

4.3 Vultures valuable for further genetic research

Returning to the research question, it was feasible to identify vultures that could be valuable for further genetic research. However, the lack of documented information posed a challenge in making definitive decisions about which vultures are truly interesting to investigate. Many founder vultures that originated from the wild are still alive and available for sample collection. Unfortunately, for a significant portion of these vultures, there is no documented information about their place of origin. This lack of data complicates their inclusion in the screening tool research. As mentioned earlier, it is crucial for the screening tool to encompass a variety of vultures originating from different geographic locations. This diversity is essential for creating genetic markers that can be associated with specific origin locations (Irizarry, et al., 2016; Templeton, 1991). With respect to the screening tool developed for distinguishing between the two sub-species of the Rüppell's griffon vulture, it is noteworthy that I managed to identify two vultures from the sub-species *Gyps rueppelli erlangeri*. It is anticipated that in conjunction with other captive vultures belonging to the sub-species *Gyps rueppelli rueppelli* will lead to the successful development of a screening tool. This tool will play a pivotal role in facilitating research focused on validating and identifying the Rüppell's griffon vulture sub-species (Verdoorn, 2004). In my study, I conducted the analysis of all samples based on mtDNA sequences. However, employing mtDNA for the creating of a screening tool may not be the most sensible choice. This is due to the inheritance pattern of mtDNA solely from the maternal ancestor (Harrison, 1989). An examination of my data revealed that numerous samples shared the exact same haplotype, which would render the development of a distinct screening tool unfeasible. The Peregrine funds, entrusted with the development of the origin screening tool, have expressed the preference for constructing it based on microsatellites. Alternatively, they could opt for a screening tool based on SNPs, as these are more abundant than microsatellites. Nonetheless, it is worth noting that SNPs are comparatively less informative, requiring a larger number of them to obtain an equivalent amount of information when compared to microsatellites (Schaid, et al., 2004). Therefore, the creation of a screening tool based on microsatellites appears to be the most advantageous choice.

Lastly, I successfully selected an individual vulture for analysis using the Oxford Nanopore Sequencer. Despite my limited choices, constrained to samples available in the Wageningen Livestock Research laboratory, I believe that the chosen vulture will provide valuable and high-quality DNA data. In summary, this section of my research underscores the feasibility of enhancing the genetic data related to the Rüppell's griffon vulture. However it also highlight the pressing needs for an extensive expansion of data collection efforts to comprehensively advance our genetic understanding of this species.

5. Conclusions and recommendations

Taking a look back at the aim of my research, enhancing our genetic understanding of the Rüppell's griffon vulture, and the associated research questions, I can conclude the following key findings:

- The genetic diversity analysis of samples from Diergaarde Blijdorp and Avifauna yielded a haplotype diversity of 0.3732 (± 0.0102) and a nucleotide diversity of 0.000488 (± 0.000527). Furthermore, regarding the population structure the analysis unveiled three previously unrecorded haplotypes. One of these haplotypes is prevalent among the samples, resulting in a limited population structure with the captive vulture population.
- A comparative analysis with the wild Rüppell's griffon vulture population revealed similarities in haplotype network and population structure. However, the genetic diversity in the wild population was notably higher than in the captive population.
- A comparative analysis with other vulture populations revealed congruences in haplotype network and population structure when compared to the Eurasian griffon vulture and Hooded vulture. However, such similarities were not observed when evaluating the White-backed and White-rumped vultures. The Rüppell's griffon vulture exhibited lower genetic diversity and genetic distance in contrast to the majority of the vulture populations.
- It is not certain if the mtDNA analysis supports the recorded maternal ancestors information in the studbook
- A significant outcome of my research is the selection of six vultures valuable for further genetic research, in addition to the identification of an individual vulture chosen for nanopore sequencing.

Overall, it can be affirmed that the primary aim of my research has been achieved. The genetic insights and conclusions derived from my study can significantly advance our genetic understanding of the Rüppell's griffon vulture.

My research offers the potential to make significant contributions to the improvement of the captive breeding program. As previously emphasized, the genetic composition of the captive population should closely mirror that of the wild population to optimize the chances of successful reintroduction. Currently, the captive population exhibits lower genetic diversity. To address this, I strongly recommend conducting a comprehensive genetic analysis of a broader selection of vultures within the captive population, spanning multiple zoos. Furthermore, it is imperative to intensify data collection efforts within the wild population. This approach will provide a more accurate depiction of the extent to which the captive population represents the genetic diversity found in the wild. Ultimately, this would help improve the captive breeding program and the conservation of the wild Rüppell's griffon vulture population.

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7. Appendixes

7.1 Appendix I: List of previously published cytb region sequences of vulture populations

For my study, 128 cytb sequence data from previous vulture studies were utilized (Table A1). These data sets were obtained from five different studies, all of which were published.

Table A1: Overview of all previously published cytb wild vulture sequences from the families Gyps africanus, Gyps bengalensis, Gyps fulvus, Gyps rueppelli and Necrosyrtes monachus.

Gen ID	Species	Source	Weblink
X86748.1	Gyps_africanus	Seibold & Helbig, 1995	https://www.ncbi.nlm.nih.gov/nuccore/X86748.1?report=fasta
EU496414.1	Gyps_africanus	Arshad, et al., 2009	https://www.ncbi.nlm.nih.gov/nuccore/EU496414.1?report=fasta
EU496413.1	Gyps_africanus	Arshad, et al., 2009	https://www.ncbi.nlm.nih.gov/nuccore/EU496413.1?report=fasta
EU496412.1	Gyps_africanus	Arshad, et al., 2009	https://www.ncbi.nlm.nih.gov/nuccore/EU496412.1?report=fasta
EU496411.1	Gyps_africanus	Arshad, et al., 2009	https://www.ncbi.nlm.nih.gov/nuccore/EU496411.1?report=fasta
EU496410.1	Gyps_africanus	Arshad, et al., 2009	https://www.ncbi.nlm.nih.gov/nuccore/EU496410.1?report=fasta
EU496409.1	Gyps_africanus	Arshad, et al., 2009	https://www.ncbi.nlm.nih.gov/nuccore/EU496409.1?report=fasta
EU496408.1	Gyps_africanus	Arshad, et al., 2009	https://www.ncbi.nlm.nih.gov/nuccore/EU496408.1?report=fasta
EU496407.1	Gyps_africanus	Arshad, et al., 2009	https://www.ncbi.nlm.nih.gov/nuccore/EU496407.1?report=fasta
EU496406.1	Gyps_africanus	Arshad, et al., 2009	https://www.ncbi.nlm.nih.gov/nuccore/EU496406.1?report=fasta
EU496405.1	Gyps_africanus	Arshad, et al., 2009	https://www.ncbi.nlm.nih.gov/nuccore/EU496405.1?report=fasta
EU496404.1	Gyps_africanus	Arshad, et al., 2009	https://www.ncbi.nlm.nih.gov/nuccore/EU496404.1?report=fasta
EU496403.1	Gyps_africanus	Arshad, et al., 2009	https://www.ncbi.nlm.nih.gov/nuccore/EU496403.1?report=fasta
EU496402.1	Gyps_africanus	Arshad, et al., 2009	https://www.ncbi.nlm.nih.gov/nuccore/EU496402.1?report=fasta
EU496401.1	Gyps_africanus	Arshad, et al., 2009	https://www.ncbi.nlm.nih.gov/nuccore/EU496401.1?report=fasta
AY987263.1	Gyps_africanus	Lerner & Mindell, 2005	https://www.ncbi.nlm.nih.gov/nuccore/AY987263.1?report=fasta
DQ908972.1	Gyps_africanus	Johnson, et al., 2006	https://www.ncbi.nlm.nih.gov/nuccore/DQ908972.1?report=fasta
X86750.1	Gyps_bengalensis	Seibold & Helbig, 1995	https://www.ncbi.nlm.nih.gov/nuccore/X86750.1?report=fasta
EU496426.1	Gyps_bengalensis	Arshad, et al., 2009	https://www.ncbi.nlm.nih.gov/nuccore/EU496426.1?report=fasta
EU496425.1	Gyps_bengalensis	Arshad, et al., 2009	https://www.ncbi.nlm.nih.gov/nuccore/EU496425.1?report=fasta
EU496424.1	Gyps_bengalensis	Arshad, et al., 2009	https://www.ncbi.nlm.nih.gov/nuccore/EU496424.1?report=fasta

EU496423.1	Gyps_bengalensis	Arshad, et al., 2009	https://www.ncbi.nlm.nih.gov/nuccore/EU496423.1?report=fasta
EU496422.1	Gyps_bengalensis	Arshad, et al., 2009	https://www.ncbi.nlm.nih.gov/nuccore/EU496422.1?report=fasta
EU496421.1	Gyps_bengalensis	Arshad, et al., 2009	https://www.ncbi.nlm.nih.gov/nuccore/EU496421.1?report=fasta
EU496420.1	Gyps_bengalensis	Arshad, et al., 2009	https://www.ncbi.nlm.nih.gov/nuccore/EU496420.1?report=fasta
EU496419.1	Gyps_bengalensis	Arshad, et al., 2009	https://www.ncbi.nlm.nih.gov/nuccore/EU496419.1?report=fasta
EU496418.1	Gyps_bengalensis	Arshad, et al., 2009	https://www.ncbi.nlm.nih.gov/nuccore/EU496418.1?report=fasta
EU496417.1	Gyps_bengalensis	Arshad, et al., 2009	https://www.ncbi.nlm.nih.gov/nuccore/EU496417.1?report=fasta
EU496416.1	Gyps_bengalensis	Arshad, et al., 2009	https://www.ncbi.nlm.nih.gov/nuccore/EU496416.1?report=fasta
EU496415.1	Gyps_bengalensis	Arshad, et al., 2009	https://www.ncbi.nlm.nih.gov/nuccore/EU496415.1?report=fasta
AY987259.1	Gyps_bengalensis	Lerner & Mindell, 2005	https://www.ncbi.nlm.nih.gov/nuccore/AY987259.1?report=fasta
DQ908979.1	Gyps_bengalensis	Johnson, et al., 2006	https://www.ncbi.nlm.nih.gov/nuccore/DQ908979.1?report=fasta
DQ908978.1	Gyps_bengalensis	Johnson, et al., 2006	https://www.ncbi.nlm.nih.gov/nuccore/DQ908978.1?report=fasta
DQ908977.1	Gyps_bengalensis	Johnson, et al., 2006	https://www.ncbi.nlm.nih.gov/nuccore/DQ908977.1?report=fasta
DQ908976.1	Gyps_bengalensis	Johnson, et al., 2006	https://www.ncbi.nlm.nih.gov/nuccore/DQ908976.1?report=fasta
DQ908975.1	Gyps_bengalensis	Johnson, et al., 2006	https://www.ncbi.nlm.nih.gov/nuccore/DQ908975.1?report=fasta
DQ908974.1	Gyps_bengalensis	Johnson, et al., 2006	https://www.ncbi.nlm.nih.gov/nuccore/DQ908974.1?report=fasta
OL962691.1	Gyps_fulvus	Davidović, et al., 2022	https://www.ncbi.nlm.nih.gov/nuccore/OL962691.1?report=fasta
OL962690.1	Gyps_fulvus	Davidović, et al., 2022	https://www.ncbi.nlm.nih.gov/nuccore/OL962690.1?report=fasta
OL962689.1	Gyps_fulvus	Davidović, et al., 2022	https://www.ncbi.nlm.nih.gov/nuccore/OL962689.1?report=fasta
OL962688.1	Gyps_fulvus	Davidović, et al., 2022	https://www.ncbi.nlm.nih.gov/nuccore/OL962688.1?report=fasta
OL962687.1	Gyps_fulvus	Davidović, et al., 2022	https://www.ncbi.nlm.nih.gov/nuccore/OL962687.1?report=fasta
OL962686.1	Gyps_fulvus	Davidović, et al., 2022	https://www.ncbi.nlm.nih.gov/nuccore/OL962686.1?report=fasta
OL962685.1	Gyps_fulvus	Davidović, et al., 2022	https://www.ncbi.nlm.nih.gov/nuccore/OL962685.1?report=fasta
OL962684.1	Gyps_fulvus	Davidović, et al., 2022	https://www.ncbi.nlm.nih.gov/nuccore/OL962684.1?report=fasta
OL962683.1	Gyps_fulvus	Davidović, et al., 2022	https://www.ncbi.nlm.nih.gov/nuccore/OL962683.1?report=fasta
OL962682.1	Gyps_fulvus	Davidović, et al., 2022	https://www.ncbi.nlm.nih.gov/nuccore/OL962682.1?report=fasta

OL962681.1	Gyps_fulvus	Davidović, et al., 2022	https://www.ncbi.nlm.nih.gov/nuccore/OL962681.1?report=fasta
OL962680.1	Gyps_fulvus	Davidović, et al., 2022	https://www.ncbi.nlm.nih.gov/nuccore/OL962680.1?report=fasta
OL962679.1	Gyps_fulvus	Davidović, et al., 2022	https://www.ncbi.nlm.nih.gov/nuccore/OL962679.1?report=fasta
OL962678.1	Gyps_fulvus		https://www.ncbi.nlm.nih.gov/nuccore/OL962678.1?report=fasta
OL962677.1	Gyps_fulvus	Davidović, et al., 2022	https://www.ncbi.nlm.nih.gov/nuccore/OL962677.1?report=fasta
OL962676.1	Gyps_fulvus	Davidović, et al., 2022	https://www.ncbi.nlm.nih.gov/nuccore/OL962676.1?report=fasta
OL962675.1	Gyps_fulvus	Davidović, et al., 2022	https://www.ncbi.nlm.nih.gov/nuccore/OL962675.1?report=fasta
OL962674.1	Gyps_fulvus	Davidović, et al., 2022	https://www.ncbi.nlm.nih.gov/nuccore/OL962674.1?report=fasta
OL962673.1	Gyps_fulvus	Davidović, et al., 2022	https://www.ncbi.nlm.nih.gov/nuccore/OL962673.1?report=fasta
OL962672.1	Gyps_fulvus	Davidović, et al., 2022	https://www.ncbi.nlm.nih.gov/nuccore/OL962672.1?report=fasta
OL962671.1	Gyps_fulvus	Davidović, et al., 2022	https://www.ncbi.nlm.nih.gov/nuccore/OL962671.1?report=fasta
OL962670.1	Gyps_fulvus	Davidović, et al., 2022	https://www.ncbi.nlm.nih.gov/nuccore/OL962670.1?report=fasta
OL962669.1	Gyps_fulvus	Davidović, et al., 2022	https://www.ncbi.nlm.nih.gov/nuccore/OL962669.1?report=fasta
OL962668.1	Gyps_fulvus	Davidović, et al., 2022	https://www.ncbi.nlm.nih.gov/nuccore/OL962668.1?report=fasta
OL962667.1	Gyps_fulvus	Davidović, et al., 2022	https://www.ncbi.nlm.nih.gov/nuccore/OL962667.1?report=fasta
OL962666.1	Gyps_fulvus	Davidović, et al., 2022	https://www.ncbi.nlm.nih.gov/nuccore/OL962666.1?report=fasta
OL962665.1	Gyps_fulvus	Davidović, et al., 2022	https://www.ncbi.nlm.nih.gov/nuccore/OL962665.1?report=fasta
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OL962663.1	Gyps_fulvus	Davidović, et al., 2022	https://www.ncbi.nlm.nih.gov/nuccore/OL962663.1?report=fasta
OL962662.1	Gyps_fulvus	Davidović, et al., 2022	https://www.ncbi.nlm.nih.gov/nuccore/OL962662.1?report=fasta
OL962661.1	Gyps_fulvus	Davidović, et al., 2022	https://www.ncbi.nlm.nih.gov/nuccore/OL962661.1?report=fasta
OL962660.1	Gyps_fulvus	Davidović, et al., 2022	https://www.ncbi.nlm.nih.gov/nuccore/OL962660.1?report=fasta
OL962659.1	Gyps_fulvus	Davidović, et al., 2022	https://www.ncbi.nlm.nih.gov/nuccore/OL962659.1?report=fasta
OL962658.1	Gyps_fulvus	Davidović, et al., 2022	https://www.ncbi.nlm.nih.gov/nuccore/OL962658.1?report=fasta
OL962657.1	Gyps_fulvus	Davidović, et al., 2022	https://www.ncbi.nlm.nih.gov/nuccore/OL962657.1?report=fasta
OL962656.1	Gyps_fulvus	Davidović, et al., 2022	https://www.ncbi.nlm.nih.gov/nuccore/OL962656.1?report=fasta
OL962655.1	Gyps_fulvus	Davidović, et al., 2022	https://www.ncbi.nlm.nih.gov/nuccore/OL962655.1?report=fasta

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OL962651.1	Gyps_fulvus	Davidović, et al., 2022	https://www.ncbi.nlm.nih.gov/nuccore/OL962651.1?report=fasta
OL962650.1	Gyps_fulvus	Davidović, et al., 2022	https://www.ncbi.nlm.nih.gov/nuccore/OL962650.1?report=fasta
OL962649.1	Gyps_fulvus	Davidović, et al., 2022	https://www.ncbi.nlm.nih.gov/nuccore/OL962649.1?report=fasta
OL962648.1	Gyps_fulvus	Davidović, et al., 2022	https://www.ncbi.nlm.nih.gov/nuccore/OL962648.1?report=fasta
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OL962646.1	Gyps_fulvus	Davidović, et al., 2022	https://www.ncbi.nlm.nih.gov/nuccore/OL962646.1?report=fasta
OL962645.1	Gyps_fulvus	Davidović, et al., 2022	https://www.ncbi.nlm.nih.gov/nuccore/OL962645.1?report=fasta
OL962644.1	Gyps_fulvus	Davidović, et al., 2022	https://www.ncbi.nlm.nih.gov/nuccore/OL962644.1?report=fasta
OL962643.1	Gyps_fulvus	Davidović, et al., 2022	https://www.ncbi.nlm.nih.gov/nuccore/OL962643.1?report=fasta
OL962642.1	Gyps_fulvus	Davidović, et al., 2022	https://www.ncbi.nlm.nih.gov/nuccore/OL962642.1?report=fasta
OL962641.1	Gyps_fulvus	Davidović, et al., 2022	https://www.ncbi.nlm.nih.gov/nuccore/OL962641.1?report=fasta
OL962640.1	Gyps_fulvus	Davidović, et al., 2022	https://www.ncbi.nlm.nih.gov/nuccore/OL962640.1?report=fasta
OL962639.1	Gyps_fulvus	Davidović, et al., 2022	https://www.ncbi.nlm.nih.gov/nuccore/OL962639.1?report=fasta
OL962638.1	Gyps_fulvus	Davidović, et al., 2022	https://www.ncbi.nlm.nih.gov/nuccore/OL962638.1?report=fasta
OL962637.1	Gyps_fulvus	Davidović, et al., 2022	https://www.ncbi.nlm.nih.gov/nuccore/OL962637.1?report=fasta
OL962636.1	Gyps_fulvus	Davidović, et al., 2022	https://www.ncbi.nlm.nih.gov/nuccore/OL962636.1?report=fasta
OL962635.1	Gyps_fulvus	Davidović, et al., 2022	https://www.ncbi.nlm.nih.gov/nuccore/OL962635.1?report=fasta
OL962634.1	Gyps_fulvus	Davidović, et al., 2022	https://www.ncbi.nlm.nih.gov/nuccore/OL962634.1?report=fasta
OL962633.1	Gyps_fulvus	Davidović, et al., 2022	https://www.ncbi.nlm.nih.gov/nuccore/OL962633.1?report=fasta
OL962632.1	Gyps_fulvus	Davidović, et al., 2022	https://www.ncbi.nlm.nih.gov/nuccore/OL962632.1?report=fasta
OL962631.1	Gyps_fulvus	Davidović, et al., 2022	https://www.ncbi.nlm.nih.gov/nuccore/OL962631.1?report=fasta
OL962630.1	Gyps_fulvus	Davidović, et al., 2022	https://www.ncbi.nlm.nih.gov/nuccore/OL962630.1?report=fasta
X86752.1	Gyps_fulvus	Seibold & Helbig, 1995	https://www.ncbi.nlm.nih.gov/nuccore/X86752.1?report=fasta
EU496438.1	Gyps_fulvus	Arshad, et al., 2009	https://www.ncbi.nlm.nih.gov/nuccore/EU496438.1?report=fasta

EU496437.1	Gyps_fulvus	Arshad, et al., 2009	https://www.ncbi.nlm.nih.gov/nuccore/EU496437.1?report=fasta
EU496436.1	Gyps_fulvus	Arshad, et al., 2009	https://www.ncbi.nlm.nih.gov/nuccore/EU496436.1?report=fasta
EU496435.1	Gyps_fulvus	Arshad, et al., 2009	https://www.ncbi.nlm.nih.gov/nuccore/EU496435.1?report=fasta
EU496434.1	Gyps_fulvus	Arshad, et al., 2009	https://www.ncbi.nlm.nih.gov/nuccore/EU496434.1?report=fasta
EU496433.1	Gyps_fulvus	Arshad, et al., 2009	https://www.ncbi.nlm.nih.gov/nuccore/EU496433.1?report=fasta
EU496432.1	Gyps_fulvus	Arshad, et al., 2009	https://www.ncbi.nlm.nih.gov/nuccore/EU496432.1?report=fasta
EU496431.1	Gyps_fulvus	Arshad, et al., 2009	https://www.ncbi.nlm.nih.gov/nuccore/EU496431.1?report=fasta
EU496430.1	Gyps_fulvus	Arshad, et al., 2009	https://www.ncbi.nlm.nih.gov/nuccore/EU496430.1?report=fasta
EU496429.1	Gyps_fulvus	Arshad, et al., 2009	https://www.ncbi.nlm.nih.gov/nuccore/EU496429.1?report=fasta
EU496428.1	Gyps_fulvus	Arshad, et al., 2009	https://www.ncbi.nlm.nih.gov/nuccore/EU496428.1?report=fasta
EU496427.1	Gyps_fulvus	Arshad, et al., 2009	https://www.ncbi.nlm.nih.gov/nuccore/EU496427.1?report=fasta
AY987261.1	Gyps_fulvus	Lerner & Mindell, 2005	https://www.ncbi.nlm.nih.gov/nuccore/AY987261.1?report=fasta
DQ908970.1	Gyps_fulvus	Johnson, et al., 2006	https://www.ncbi.nlm.nih.gov/nuccore/DQ908970.1?report=fasta
DQ908969.1	Gyps_fulvus	Johnson, et al., 2006	https://www.ncbi.nlm.nih.gov/nuccore/DQ908969.1?report=fasta
DQ908968.1	Gyps_fulvus	Johnson, et al., 2006	https://www.ncbi.nlm.nih.gov/nuccore/DQ908968.1?report=fasta
DQ908967.1	Gyps_fulvus	Johnson, et al., 2006	https://www.ncbi.nlm.nih.gov/nuccore/DQ908967.1?report=fasta
DQ908966.1	Gyps_fulvus	Johnson, et al., 2006	https://www.ncbi.nlm.nih.gov/nuccore/DQ908966.1?report=fasta
DQ908965.1	Gyps_fulvus	Johnson, et al., 2006	https://www.ncbi.nlm.nih.gov/nuccore/DQ908965.1?report=fasta
EU496454.1	Gyps_rueppelli	Arshad, et al., 2009	https://www.ncbi.nlm.nih.gov/nuccore/EU496454.1?report=fasta
EU496453.1	Gyps_rueppelli	Arshad, et al., 2009	https://www.ncbi.nlm.nih.gov/nuccore/EU496453.1?report=fasta
EU496451.1	Gyps_rueppelli	Arshad, et al., 2009	https://www.ncbi.nlm.nih.gov/nuccore/EU496451.1?report=fasta
DQ908973.1	Gyps_rueppelli	Johnson, et al., 2006	https://www.ncbi.nlm.nih.gov/nuccore/DQ908973.1?report=fasta
EU496460.1	Necrosyrtes_monachus	Arshad, et al., 2009	https://www.ncbi.nlm.nih.gov/nuccore/EU496460.1?report=fasta
EU496459.1	Necrosyrtes_monachus	Arshad, et al., 2009	https://www.ncbi.nlm.nih.gov/nuccore/EU496459.1?report=fasta
AY987258.1	Necrosyrtes_monachus	Lerner & Mindell, 2005	https://www.ncbi.nlm.nih.gov/nuccore/AY987258.1?report=fasta
DQ908980.1	Necrosyrtes_monachus	Johnson, et al., 2006	https://www.ncbi.nlm.nih.gov/nuccore/DQ908980.1?report=fasta

X86756.1	Necrosyrtes_monachus	Seibold & Helbig, 1995	https://www.ncbi.nlm.nih.gov/nuccore/X86756.1?report=fasta

7.2 Appendix 2: Agarose gel mtDNA extracts

Figure A1 illustrates that the mtDNA extracts were of good quality, as the samples did not migrate in the agarose gel.

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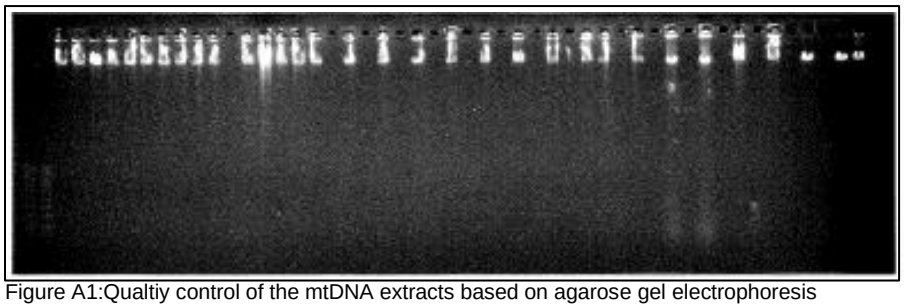

Figure A1:Qualtiy control of the mtDNA extracts based on agarose gel electrophoresis

7.3 Appendix 3: Agarose gel PCR

Figure A2 and A3 illustrates that the PCR products were of good quality, as the samples did not migrate in the agarose gel.

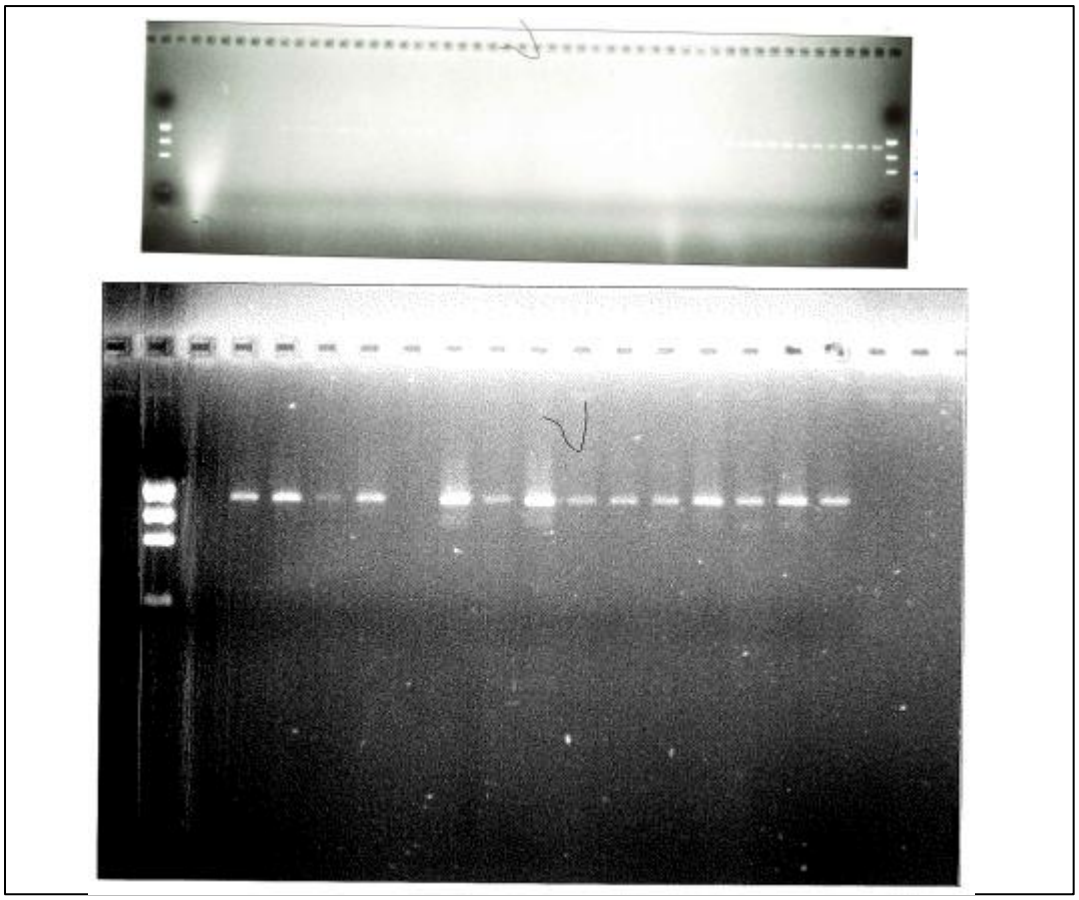

Figure A2:Qualtiy control of the PCR cleanup based on agarose gel electrophoresis

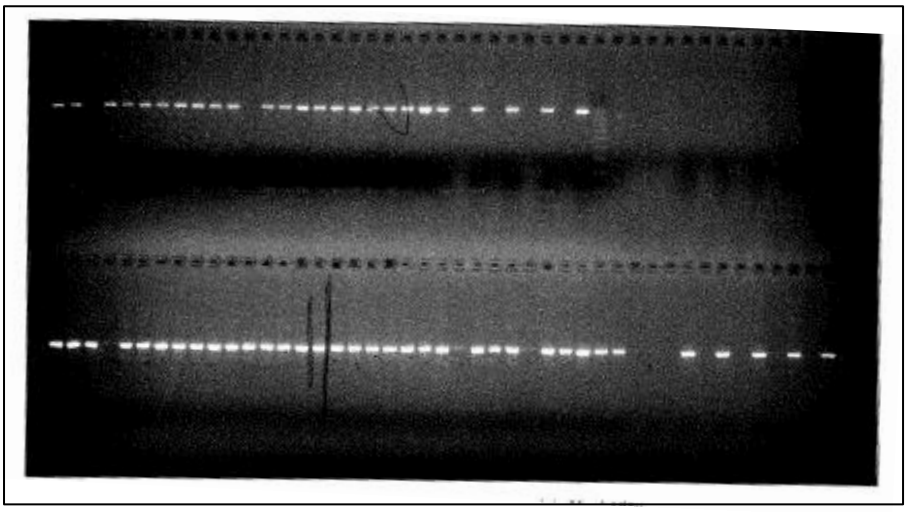

Figure A3:Qualtiy control of the PCR quality check based on agarose gel electrophoresis

7.4 Appendix 4: Purification protocol

Sequencing protocol version: 20-06-23
Purification of PCR products

The PCR product should be cleaned from primers, to prevent the sequencing reaction to start at both ends.
This purification is done by running columns as described below.

Use Millipore PCR cleanup vacuum system (Multiscreen_PCR vacu 030)

1. Load PCR reactions into the Multiscreen_PCR plate.
2. Place the Multiscreen_PCR plate on top of the Vacuum manifold.
3. Apply vacuum at 500 mmHg for 5 minutes or until the wells have emptied.
   Allow 30 extra seconds under vacuum after the well appears empty to be sure all liquid has been filtered. The filter appears shiny even after they are dry.
4. Load filter with 35 µl ddH_2O and apply the vacuum again for 5 minutes or until the wells have emptied. (repeat this step)
5. After vacuum filtration is complete, remove the plate from the manifold, blot from underneath with paper towels and add 12µl MQ (equal to start volume of the PCR-reactions) to each well.
6. Mix samples vigorously on a plate shaker for 5 minutes
7. Retrieve purified PCR product from each well by pipetting.
8. Check quality and quantity on agarose gel.
   For quantification, use 5 µl EZ load precision as a marker.
   Load 2 µl purified PCR product on 1,5% Agarose gel.

7.5 Appendix 5: Clean-up protocol

Sequencing protocol version: 20-06-23
Sequencing Reaction Clean-Up

Precipitation

10 µl seq. reaction
1 µl NaAc-EDTA
34 µl EtOH (-20 degrees)
45 µl mix this by vortexing

Incubate 30 minutes on ice

Centrifuge 30 minutes 3000g

Centrifuge up side down 1 minute 700g

Dissolve the sample in 10 µl MQ

Pipet 10 µl of formamide in a barcode plate and add 2 µl sample.

Run the barcode plate with samples in the ABI3730.

7.6 Appendix 6: Complete captive Rüppell's griffon vulture population data set

Table A2: Overview of all captive Rüppell's griffon vulture samples used in the DNA extraction and sequencing. Includes sample ID, studbook ID, type of data, concentration in nanogram per µL, there 260/280 value and 260/230 value and data usage in my research.

Sample	Sample ID	Studbook ID	Type of data	Concentration nanogram/µL	260/ 280	260/ 230	Data used in research
G 1.1	Z11073	1193	Blood	202	1,9	2	Yes
G 1.2	Z19084	1226	Blood	276	1,9	2,4	Yes
G 1.3	409951	1089	Blood	514	1,9	2,3	Yes
G 1.4	Z12084	1202	Blood	362	1,9	2,3	Yes
G 1.5	Z2221	1339	Blood	436	1,9	2,3	Yes
G 1.6	Z2257	1342	Blood	624	1,9	2,4	Yes
G 1.7	409952	1090	Blood	320	1,9	2,3	Yes
G 1.8	409955	1093	Blood	302	1,9	2,3	Yes
G 2.1	NL 24.0 AO 6223 22 3	1341	Blood	212	1,9	2,3	Yes
G 2.2	968000004762771	1136	Blood	79,2	1,9	2,4	No
G 2.3	528224001049173	1267	Blood	192	1,9	2,3	Yes
G 2.4	968000004767705	1155	Blood	141	1,9	2,3	Yes
G 2.5	982009104243060	1149	Blood	164	1,9	2,3	Yes
G 2.6	528210000820528	1052	Blood	208	1,9	2,3	Yes
G 2.7	528224001049169	1268	Blood	208	1,9	2,3	Yes
G 2.8	968000004465545	1152	Blood	238	1,9	2,3	Yes
G 2.9	968000004819681	1141	Blood	58,4	1,9	2,2	Yes
G 2.10	965000000090575	1186	Blood	68,4	1,9	2,3	Yes
G 2.11	968000004756542	1154	Blood	69,4	1,9	2,3	Yes
G 2.12	528210002348114	1197	Blood	142	1,9	2,3	Yes
G 2.13	900200000882727	1269	Blood	90,6	1,9	2,3	Yes
G 2.14	968000004754055	1138	Blood	120	1,9	2,3	Yes
G 2.15	968000004737461	1139	Blood	214	1,9	2,3	Yes
G 2.16	968000004268327	1143	Blood	144	1,9	2,3	Yes
G 2.17	Z2023	1305	Blood	195	1,9	2,3	No
G 2.18	Z20416	1301	Blood	11,2	1,9	2	No
G 2.19	Z19083	1224	Blood	224	1,9	2,4	Yes
G 2.20	Z209	1303	Blood	171	1,9	2,3	No
BR1	Z209	1303	Blood	0,84	2,4	0,6	Yes
BR2	Z20416	1301	Blood	3,5	1,9	1,2	Yes
BR3	Z2023	1305	Blood	1,0	2,7	0,7	Yes
BR4	Z19083	1224	Blood	0,4	4,1	0,4	No
R1	Z20416	1301	Feather	13,0	1,9	1,2	No
R2	Z2023	1305	Feather	12,3	1,8	1,3	No
R3	Z19083	1224	Feather	13,0	1,9	0,8	No
R4	Z2023	1305	Feather	11,3	2,0	1,3	No
R5	Z209	1303	Feather	8,2	1,9	1,2	No
R6	Z2022	Unknown	Feather	7,3	1,9	0,9	No

7.7 Appendix 7: RStudio code haplotype network

```
library("ape")
library("pegas")
setwd("~/MSc Thesis/Data")
data<-read.dna("name_file.fas", format="fasta")
dataAli<-clustal(data)
checkAlignment(dataAli)
dataHaplo<-haplotype(dataAli)
dataHaplo
dataHaplo<-sort(dataHaplo, what = "labels")
dataNet<-haploNet(dataHaplo)
countHap <- function(hap = h, dna = x){
  with(
    stack(setNames(attr(hap, "index"), rownames(hap))),
    table(hap = ind, pop = attr(dna, "dimnames")[[1]][values])
  )
}
write.table(countHap (dataHaplo, dataAli),file="FILE_NAME.txt", sep="\t", quote=FALSE)
pdf(file="~/MSc Thesis/Data/haploNet_Gyps_rueppelli_captive.pdf", width = 8, height = 15,
pointsize = 10)
plot(dataNet, size=attr(dataNet, "freq"), scale.ratio=0.2, pie=countHap(dataHaplo, dataAli),
show.mutation=3)
legend("bottomleft", colnames(countHap(dataHaplo, dataAli)),
col=rainbow(ncol(countHap(dataHaplo, dataAli))), pch=19, ncol=2)
dev.off()
hapDiv<-hap.div(dataAli, variance=TRUE)
pi<-nuc.div(dataAli, variance = TRUE)
tajima<-tajima.test(dataAli)
hapDiv
pi
Tajima
```

7.8 Appendix 8: Phylogenetic trees of different vulture populations

Figure A4 and A5 illustrate the individual phylogenetic trees of all the vulture populations used in this study, encompassing captive and wild Rüppell's griffon vultures, White-rumped vulture, Eurasian griffon vulture, White-backed vulture and Hooded vultur

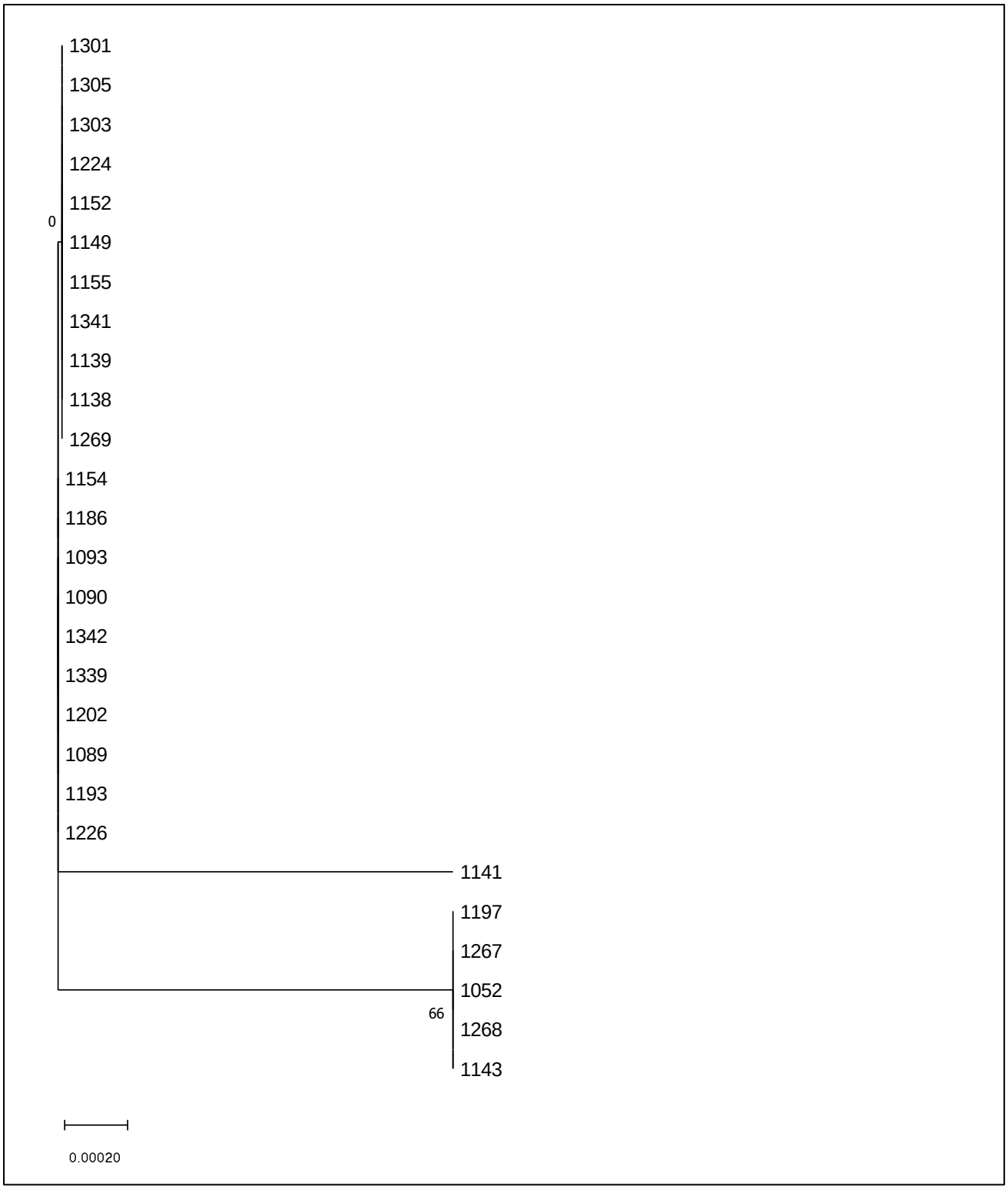

Figure A4: Phylogenetic tree depicting the genetic relationships among all haplotypes of captive Rüppell's griffon vulture population. The tree is constructed based on the variability of the cytb region sequences using the ML method. Genetic distances are included, and bootstrap branch support values are assigned to each branch.

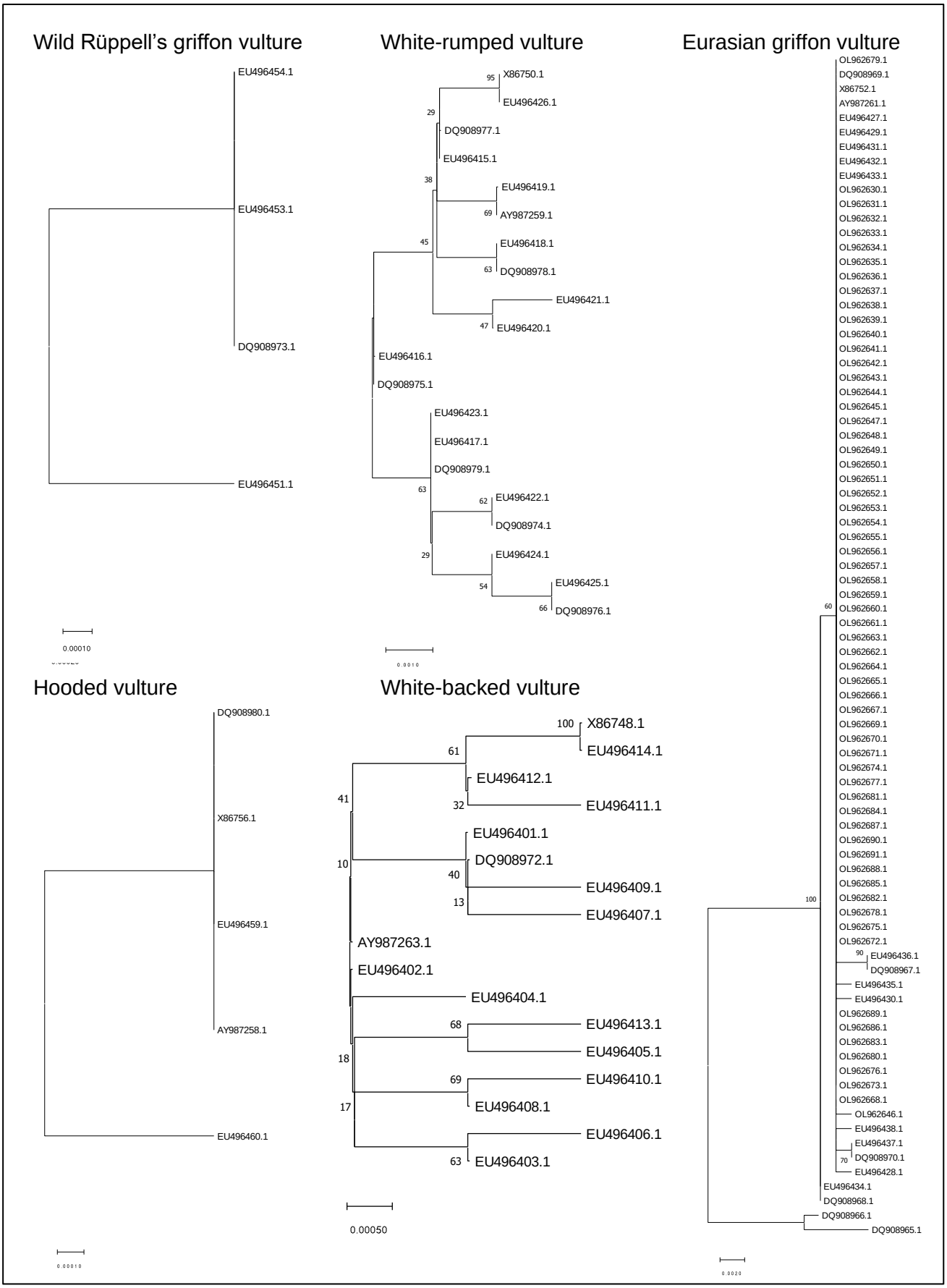

Figure A5: Phylogenetic trees depicting the genetic relationships among all haplotypes of the various vulture populations used in this study, encompassing wild Rüppell's griffon vultures, White-rumped vulture, Eurasian griffon vulture, White-backed vulture and Hooded vultur. The tree is constructed based on the variability of the cytb region sequences using the ML method. Genetic distances are included, and bootstrap branch support values are assigned to each branch.

7.9 Appendix 9: Haplotypes of all captive vulture samples

Table A3: mtDNA haplotypes based on the cytb region of the captive Rüppell's griffon vultures from Diergaarde Blijdorp and Avifauna. Includes sample ID, sex, place of origin, current residence location, and haplotype.

Sample ID	Sex	Place of origin	Residence	Cytb haplotype
1052	Female	Possibly Ivoorkust	Avifauna	12A 698T
1089	Male	Unkown	Blijdorp	12G 698T
1090	Male	Unkown	Blijdorp	12G 698T
1093	Female	Unkown	Blijdorp	12G 698T
1138	Male	Unkown	Avifauna	12G 698T
1139	Male	Unkown	Avifauna	12G 698T
1141	Male	Unkown	Avifauna	12G 698C
1143	Male	Unkown	Avifauna	12A 698T
1149	Male	Unkown	Avifauna	12G 698T
1152	Male	Unkown	Avifauna	12G 698T
1154	Male	Unkown	Avifauna	12G 698T
1155	Male	Unkown	Avifauna	12G 698T
1186	Female	Zlin Lesna	Avifauna	12G 698T
1193	Female	Blijdorp	Blijdorp	12G 698T
1197	Female	Avifauna	Avifauna	12A 698T
1202	Female	Blijdorp	Blijdorp	12G 698T
1224	Male	Unkown	Blijdorp	12G 698T
1226	Male	Unkown	Blijdorp	12G 698T
1267	Female	Avifauna	Avifauna	12A 698T
1268	Female	Avifauna	Avifauna	12A 698T
1269	Female	Banham	Avifauna	12G 698T
1301	Male	Avifauna	Blijdorp	12G 698T
1303	Male	Blijdorp	Blijdorp	12G 698T
1305	Male	Blijdorp	Blijdorp	12G 698T
1339	Female	Blijdorp	Blijdorp	12G 698T
1341	Female	Blijdorp	Avifauna	12G 698T
1342	Female	Blijdorp	Avifauna	12G 698T