Conservation genetics of the Hooded vulture

Necrosyrtes monachus

By

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Submitted in fulfilment of the academic requirements for the degree of

MAGISTER SCIENTIAE

In the Discipline of Genetics

School of Life Science

College of Agriculture, Engineering and Science

University of KwaZulu-Natal

Pietermaritzburg Campus

2023

Supervisor: Prof Sandi Willows-Munro

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Conference attended

Conferences I attended and presented research.

1. 28th International Ornithological Conference (IOC) (15 – 19 August 2022)
   First record of hybridization in the vulture genus *Gyps*
   Rynhardt Le Roux, Kerri Wolter, Sandi Willows-Munro

2. Biodiversity and Evolution Cluster weekly seminar (9 November 2022)
   Conservation genetics of Hooded vulture *Necrosyrtes monachus*
   Rynhardt Le Roux, Lindy J. Thompson, Bettine van Vuuren, Sandi Willows-Munro

Abstract

African vulture species have experienced rapid population declines, due to many anthropogenic threats. Hooded vultures are no exception and have experienced dramatic declines and are now listed as Critically Endangered on the IUCN Red Data list. Two subspecies of Hooded vulture have been described : *Necrosyrtes monachus monachus* which occurs in West Africa and *Necrosyrtes monachus pileatus* which occurs in East and southern Africa. The two subspecies differ in their feeding behaviour and morphology supporting the validity of the subspecies status. However, the validity of this taxonomic grouping is still being questioned. Clarifying the taxonomic status of the subspecies is important as if the two subspecies are genetically distinct then they should not be managed as a single species and current conservation policies would need to be updated. In addition, there is limited information available on many aspects of Hooded vulture life history including the factors affecting reproduction in the wild. In Chapter 2 I use microsatellite data collected from across the distributions of the two subspecies and Approximate Bayesian Computation (ABC) to test the hypothesis that the two subspecies are genetically distinct and should be elevated to separate species. In Chapter 3 I examine the genetic variation present in the South African Hooded vulture population. This population only includes 100-200 individuals and is at the edge of the southern range of the species. The conservation value of peripheral populations is debatable as these populations are often isolated and smaller with genetic drift and inbreeding leading to reduced genetic variability. In contrast, studying the genetic diversity in range-edge populations is important for understanding range shifts and adaptive capacity under climate change. These edge populations could potentially also retain unique genetic diversity which helps with the adaptation of species to different environments. Vulture colonies act as "food finding information hubs" allowing for the exchange of information regarding potential food resources. This explains, in part, the high-levels of relatedness often found within colonies as close relatives are more likely to tolerate the cost of sharing food by increasing their inclusive fitness. Hooded vultures are tree nesters with a single breeding pair per tree. In Chapter 4 I use the genetic data to test if individuals nesting close to each other are closely related and if the same individuals use the same nest over multiple years. The analyses conducted in Chapter 2 did not support the existence of the two subspecies classification, due to different demographic events experienced between the two groups. The next factor indicating that there is no subspeciation is the contemporary gene flow that is still seen between the population (m = 0.188) and the little variance seen between the two subspecies (11.9%). Structure analysis also does not support the formation of two distinct subspecies. Thus, this study supports the claim made by Mundy 2021 that it is size cline and not speciation. In Chapter 3 the genetic data did not support the hypothesis that the small South African population was genetically depauperate, instead the results show that the South African population contained similar levels of genetic diversity (H_o = 0.495) to that recorded for the Ghanaian population (H_o = 0.315) where Hooded vultures are more abundant. Levels of heterozygosity were similar to those recorded for other species of Old World vultures such as Cape Vultures (*Gyps coprotheres,* H_o = 0.380), and Bearded vultures (*Gypaetus barbatus* H_o = 0.400 – 0.480), but differed from the Griffon Vulture (*Gyps fulvus* H_o = 0.530 – 0.600) found in Europe. Worryingly, both populations of Hooded vultures show elevated levels of inbreeding and relatedness. The bottleneck analysis for both populations show no sign of a recent bottleneck and a normal L shaped distribution for both populations. In Chapter 4 breeding pairs were not found to reuse the same nests over multiple years. A negative correlation was seen between genetic distance and geographical distance (R^2 = 0.0117; p-value = 0.012) the closer related individuals thus tend to nest further away from each other. The spatial autocorrelation shows a positive correlation between genetic and geographical distance between distance classes 8 km – 16km, 32 km – 40km and then between 88 km – 112km, but no clear support for increased relatedness between closer nesting individuals. Thus no support is seen for the formation of loose colonies to function as food finding information sharing hubs. African vultures are facing a number of challenges and most species are considered of conservation concern. Despite this limited genetic data is available for many species. This study aimed to fill this knowledge gap by generating and analysing microsatellite data for the Critically Endangered Hooded vulture to answer a number of key hypotheses. As such this study makes an important contribution towards the conservation of Hooded vultures across Africa.

PREFACE

The data described in this thesis were collected in the Conservation Genetics Lab at the School of Life Sciences, University of KwaZulu-Natal, Pietermaritzburg, under the supervision of Professor Sandi Willows-Munro and co-supervision of Dr Lindy J. Thompson and Professor Bettine van Vuuren.

This thesis, submitted for the degree of Master of Science in the College of Agriculture, Engineering and Science, University of KwaZulu-Natal, School of Life Sciences, Pietermaritzburg campus, represents original work by the author and has not otherwise been submitted in any form for any degree or diploma to any University. Where use has been made of the work of others, it is duly acknowledged in the text.

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Rynhardt Le Roux

July 2023

I certify that the above statement is correct and as the candidate's supervisor, I have approved this thesis for submission.

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Professor Sandi Willows-Munro

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DECLARATION 1 - PLAGIARISM

I, Rynhardt Le Roux, declare that.

1. The research reported in this thesis, except where otherwise indicated, is my original research.
2. This thesis has not been submitted for any degree or examination at any other university.
3. This thesis does not contain other persons' data, pictures, graphs, or other information unless specifically acknowledged as being sourced from other persons.
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Signed:  .....

Rynhardt Le Roux

July 2023

Acknowledgements

I would like to extend my gratitude to the following people and organisations for your assistant throughout this journey.

I would first like to express my deepest gratitude to my supervisor, Prof Sandi Willows-Munro for helping me undertake this study and for her continued support throughout this project. Thank you for all the opportunities you have provided me as a postgrad student with this project and other projects.

My co-supervisor Bettine van Vuuren for her continuous support throughout my academic journey. Thanks for your patience and encouragement on days when I did not believe in myself. Without you, I would not be where I am today. Then also my other co-supervisor Lindy Thompson for your endless support and for providing me with samples. Thank you for providing me with the opportunity to research these amazing animals.

The National Research Foundation for providing funding for this project through the general Master's Scholarship. The funding helped make the thesis what it is today.

I would then like to thank the various museums for the samples they provided me. Allowing me to conduct a big portion of this project. Then a thanks to the Centre of High-Performance Computing (CHPC) for their resources allocated to me. Then we are grateful to the landowners who granted us access to conduct our fieldwork. John P. Davies for his assistance in the field while collecting these samples and Justus Deikumah for providing me with samples from Ghana**.**

I would then like to thank Courtneë van der Byl, Melanie Streicher and Mahlatse Mashaphu for their assistance and training in the lab at the start of this project. I would then like to also thank Dr Isa-Rita Russo for the training that allowed me to do the ABC modelling. Then assistance with editing and general discussion about my topic I would like to thank Dr Celine Hanzen, and Matthew Adair. Thank you for all your help with editing maps and other chapters. Finally, thanks for also being there to listen to me complained a lot about things out of your control and for the advice you gave me to get through it.

To my friends Fatima Hoosen, Cara-Ann Long and Gizelle van der Westhuyzen thank you for the support and motivation. Without you, I would have probably given up a long time ago. Thank you for believing in me every step of the way. Fatima without your assistance thought out my academic career I would not have succeeded, and for that I am forever grateful.

Then to my family a big thanks for your unconditional support from the first day of university. To my grandparents and parents, Rina van Rensburg, Jan Willem Van Rensburg, Jan Le Roux and Chrisalda Le Roux, thank you for all the opportunities you have created for me thought my life. Without you I would not be who I am today, thank you for the guidance and love you have given me. Then finally to my brother and sister Jean-Pierre Le Roux and Alicia Le Roux, thanks for always being there for me when I needed you.

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Chapter 1

Literature Review

Abstract

The Hooded vulture (*Necrosyrtes monachus*, Temminck, 1823) is an Old-World vulture distributed across most of Africa, with the exception of very dry deserts of South and North Africa and the forests of Central Africa. This species consists of two subspecies; *Necrosyrtes monachus monachus* (Temminck, 1823), found in North-West Africa and *Necrosyrtes monachus pileatus* (Burchell 1824) distributed in the South-East. These subspecies differ slightly morphologically in terms of body measurements. This has prompted much debate surrounding the validity of this taxonomic classification. Some authors consider this a monotypic species, with the differences seen in size representing a cline. This obligate scavenger performs a critical ecological service and has suffered severe population declines in most of its pan-African distribution. These severe declines in population size have resulted in the species being up-listed to Critically Endangered in 2015. This decline in population size has resulted from various anthropogenic factors, such as direct and indirect poising, human persecution, habitat destruction, and the use of traditional medicine and bush meat. The trade of Hooded vulture parts in West Africa is a very lucrative business; individuals can earn up to $7 880 for one Hooded vulture egg. Habitat destruction in most West African countries has resulted in nesting trees being removed to prevent birds from nesting near residential areas or for logging. Food availability also affects the population size of Hooded vultures, as in West Africa, a decline in Hooded vulture population sizes was observed when better waste management was implemented. Microsatellite markers allow for high levels of resolution when inferring changes in the genetic diversity of populations and are routinely used to infer population structure and demographic changes. Approximate Bayesian Computation (ABC) can be implemented to infer changes in population demography using simulations. This study aims to clarify the taxonomic status of the two Hooded vulture subspecies and examine the genetic diversity of the species. The second aim is to assess the genetic health and diversity of the South African edge population and compare it to a core population in Ghana. Then lastly, this study also aims to provide important insight into the population dynamics of Hooded vultures in South Africa.

Introduction

The Hooded vulture (*Necrosyrtes monachus*, Temminck, 1823) is endemic to Africa and occurs over most parts of Sub-Sahara Africa, except for arid areas in North and Southern Africa or heavily forested areas in central Africa (Figure 1.1; Mundy et al., 1992; Odino et al., 2014; Ogada and Buij, 2011). Hooded vultures are a solitary species that can be identified by their small stature and scruffy appearance with a height of 67-70 cm and with a wingspan of 1.7 m. They are primarily brown with a long thin bill, a bare crown, face and fore neck, conspicuous earholes, and a downy nape and hindneck (McLachlan and Liversidge, 2016; Mundy et al., 1992). When perched, they are usually hunched with wings drooping. There is no sexual dimorphism; juveniles are identifiable by their pale blue face and hood with short down that is dark brown rather than beige (McLachlan and Liversidge, 2016; Mundy et al., 1992). Hooded vultures are obligate scavengers but have also been observed congregating in large numbers to feed on emerging insects (McLachlan and Liversidge, 2016; Mundy et al., 1992; Thompson et al., 2020).

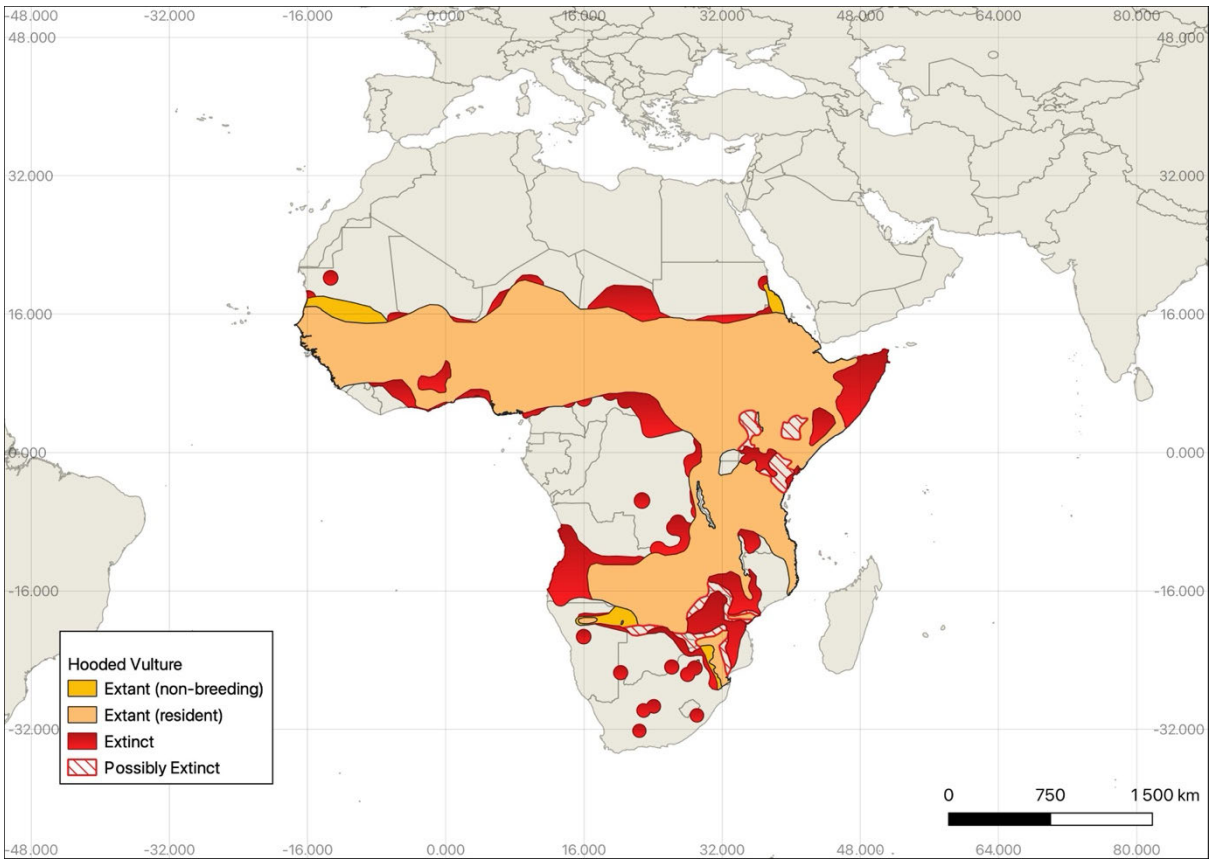

Figure 1.1: Distribution map of Hooded Vultures (*Necrosyrtes monachus*, Temminck, 1823). Redrawn from BirdLife International 2022.

In West Africa, the primary breeding season is November and July, although they are known to breed throughout the year in some areas. In northeast Africa, the breeding season is between October and July and in southern Africa, breeding takes place from May to December (Dabone et al., 2016; Roche, 2006). Hooded vultures are arboreal nesters and favour large trees such as jackal-berry (*Diospyros mespiliformis*) and common cluster fig (*Ficus sycomorus*) found near water (Ayuba et al., 2020; Daneel, 1984; Mundy et al., 1992; Roche, 2006; Thompson et al., 2017a).

Vultures play a critical role in the ecosystem as they provide vital environmental sanitation services (Ogada et al., 2012, 2016; Buechley and Şekercioğlu, 2016; Kankam and Abukari, 2020; Thompson et al., 2020). As obligate scavengers, they remove carrion, meat waste and bones from the environment (Ogada et al. 2012, Buechley & Şekercioğlu 2016, Kankam & Abukari 2020). The presence of vultures may also reduce the risk of disease outbreaks (Van den Heever et al., 2021). An example of the vital role vultures play was seen in South Asia, where vulture numbers dramatically decreased due to the use of the veterinary drug Diclofenac. Without vultures to remove carcasses, populations of other scavengers, such as feral dogs (Henriques et al., 2018; Kankam and Abukari, 2020; Ogada et al., 2016, 2012) increased, which in turn led to a significant spike in rabies infections that fast became a very expensive public health crisis (Kankam and Abukari, 2020; Markandya et al., 2008).

Distribution and population size

Although a common species in West Africa (Henriques et al., 2018; Jallow et al., 2022, 2016), this species is rare in southern Africa, with low population densities in the Okavango Delta, Hwange and regions along the Zambezi River, Zimbabwe and the Kruger National Park, South Africa (Monadjem et al., 2004; Mundy et al., 1992; Ogada and Buij, 2011; Roche, 2006). In 2010, this species was listed as Least Concern on the IUCN Red List of Threatened Species, but in 2015 this species was up-listed to Critically Endangered due to a dramatic decrease in population numbers (BirdLife International, 2017; Nosazeogie et al., 2018; Odino et al., 2014; Ogada et al., 2016; Ogada and Buij, 2011). Worryingly, the decline has been recorded throughout the distribution of the species (Barlow et al., 2021; Henriques et al., 2018; Ogada et al., 2012). For example, a 62% decrease in population size was seen in the Masai Mara National Reserve in Kenya between 1976 and 2005, and only three vultures were spotted in a 7 200 km radius in the same area in 2010 (Odino et al., 2014). A 15% reduction in the amount of carrion consumed by Hooded vultures in the Horn of Africa has been linked to reduced

numbers of vultures in the area (Buechley et al. 2022). A survey in 2018 only recorded the presence of one Hooded vulture and no other vultures in Nigeria (Ringim et al., 2022). In Kampala, Uganda, the population is unstable, increasing by 48% between 1973-2005 and later experiencing a 78% decline between 2007-2014 (Kibuule, 2016; Roberts, 2013; Ssemmanda, 2006; Ssemmanda and Pomeroy, 2010).

In contrast, in some regions Hooded vulture populations have not decreased significantly. For example, in The Gambia, Guinea-Bissau and Ghana, Hooded vultures have taken advantage of anthropogenic food sources such as rubbish dumps, abattoirs, and sewage plants (Odino et al., 2014). Guinea-Bissau is believed to be a stronghold for this species as this country has the highest density of individuals (Barlow et al., 2021; Barlow and Fulford, 2013; Gbogbo et al., 2016; Henriques et al., 2018; Jallow et al., 2016; Ogada et al., 2012) and recent counts have shown the population to be 43 000 individuals strong (Henriques et al., 2017). In contrast, the population in southern Africa is small and contains only around 100-200 mature individuals (Taylor et al., 2015). Current estimates suggest that there are approximately 131 000 Hooded vultures in Africa (BirdLife International, 2022).

Anthropogenic threats faced by Hooded vultures

Several threats are responsible for the widespread decline of African vultures. These include but are not limited to poisoning (direct and indirect), the poaching of vultures for traditional medicine and consumption, habitat loss, diminished food availability, interference at nesting sites by humans and collisions with power lines (Daboné et al., 2019; Ogada et al., 2012; Thiollay, 2007, 2006; Thompson et al., 2020; Tucker et al., 2019). Poisoning of vultures is the leading cause of population decline (Brink et al., 2020; Buechley and Şekercioğlu, 2016; Ogada et al., 2012; Thiollay, 2007, 2006). Poisoning occurs for various reasons; these reasons include direct poisoning for poaching of vultures for the traditional medicine trade and sentinel poisoning of vultures by poachers to prevent the signalling of a poaching event that occurred. Lastly, indirect poisoning by farmers when carcases are laced with poisoned to control problem carnivores and herbivores that decimate farmers' livestock and crops (Brink et al., 2020; Ogada et al., 2012; Thiollay, 2007, 2006).

Vultures are killed for belief-based trade (Daboné et al., 2019; McKean et al., 2018; Ogada et al., 2012; Saidu and Buij, 2018). This belief-based trade includes using vulture parts in traditional medicine in southern Africa (Daboné et al., 2019; McKean et al., 2018; Ogada et al., 2016, 2012; Saidu and Buij, 2018). Vulture parts are sold in large numbers in southern and

West Africa for their perceived value for treating various illnesses (Daboné et al., 2019; McKean et al., 2018; Saidu and Buij, 2018). Apart from traditional medicine, vultures are also used to protect people against witchcraft; the heads of Hooded vultures are thought to aid in protection against witches, and the whole body is used for good fortune (Ogada and Buij, 2011; Saidu and Buij, 2018). Trading with Hooded vulture products for belief-based use is a profitable business. In West African countries, a dealer from Nigeria was willing to pay US$ 7 880 (R 58 218.23) for four Hooded vulture eggs in 2009, making it more likely that there is still widespread capture of these animals for this purpose (Ogada & Buij 2011).

The trade of Hooded vultures as a food source in the bushmeat trade is also common in West Africa (Buij et al., 2016; Ogada et al., 2016; Ogada and Buij, 2011). This over-exploitation of Hooded vultures in this region could be due to their close association with humans in urban areas (Ogada and Buij, 2011). In southern Africa, Hooded vultures are found almost exclusively in conservation areas, and their consumption as bushmeat is rare. The trade of bushmeat, especially with vultures, is so high in West Africa that a trade route between Niger and Nigeria was formed specifically for the trade of smoked vulture meat (Ogada et al., 2016; Ogada and Buij, 2011). West African bushmeat markets sell 975-1462 Hooded vultures annually (Buij et al., 2016). Other vultures such as the White-backed vulture (*Gyps africanus*), Rüppell's griffon (*Gyps rueppelli*), Palm-nut vultures (*Gypohierax angolensis*) and Lappet-faced vultures (*Torgos tracheliotos*) are also on sale in these markets in large quantities (Buij et al., 2016). The meat trade contributes significantly to population declines (Buij et al., 2016).

Loss of available habitat, removal of nest sites and nest disturbance is also a critical factor that comes into play when investigating population decline in Hooded vultures (Daboné et al., 2019; Ogada et al., 2012; Thiollay, 2007). In the Garango area in Burkina Faso, a study over two years investigating the human impacts on Hooded vulture breeding success found that a quarter of the 64 breeding attempts failed due to human activities such as the removal of eggs and logging of trees close to the nests (Daboné et al., 2019). Similarly, in southern Africa, nest disturbance by geese and monkeys also negatively impacted breeding success (Thompson et al., 2019, 2017b).

The impact of food availability and human perception on Hooded vultures

Food availability for Hooded vultures may influence population numbers (Ogada et al., 2012; Teklemariam and Afework, 2021; Thiollay, 2007). In West Africa, these scavengers feed in and around urban areas near dumpsites. In some countries like Guinea Bissau and Guinea, these

birds are found between humans feeding on street corners (Mullié et al., 2017). Despite the ability to exploit new food sources in urban areas in recent years, an 85% decrease has been seen in these West African populations, and this may be due to better waste management in these areas (Gbogbo and Awotwe-Pratt, 2008; Mullié et al., 2017; Ogada et al., 2012). In The Gambia, these birds have been observed exploiting marine carrion on beaches and at fishery landing sites; this population has also been seen feeding on items like owl pellets, oil palm kernels and road-killed frogs (Barlow, 2004; Barlow et al., 2022; Barlow and Brohaugh, 2022; Mikkola and Barlow, 2022). In southern Africa, these birds are known to follow wild dog (*Lycaon pictus*) packs, where they would then scavenge food from the pack's hunt (Reading et al., 2018). Hooded vultures have also been observed consuming wild dog faeces (Reading et al., 2018). In vultures, coprophagy has only otherwise been recorded in New World species, and it has been suggested to occur so vultures can gain nutrients they struggle to find elsewhere (Reading et al., 2018). This observation in southern Africa may indicate that they cannot obtain all their needed nutrients from scavenging, or how flexible Hooded vultures are in their food choices. This can also be an indication of how they are able to adapt to what resources are found in the local area.

Understanding what anthropogenic threats are faced by these vultures is not the only way to conserve the species. Understanding the knowledge and perceptions that members of the community have about vultures is of great importance, as local people can directly impact these populations. In Nigeria, 46% of people perceive vultures as evil (Pam et al., 2021). In many countries, the need for conservation and the decline in the vulture populations have been seen by local communities (Mdhlano et al., 2019; Owolabi et al., 2021; Pam et al., 2021). Unfortunately, the critical role vultures play in an ecosystem is not fully understood by everyone; thus, researchers suggest improving public awareness and educational programs on the importance and conservation of vultures (Mdhlano et al., 2019; Nosazeogie et al., 2018; Owolabi et al., 2021)

Phylogeny and taxonomy of Hooded vultures

Two subspecies of Hooded vultures have been described. *Necrosyrtes monachus monachus* (Temminck, 1823) occurs in parts of West Africa and is distributed in the south of Mauritania, Senegal through Niger and Chad to west Sudan, South Sudan, and northern Uganda (Barlow et al., 2021; Mundy et al., 1992). *Necrosyrtes monachus pileatus* (Burchell 1824) occurs in East and southern Africa with its distribution extending into east Sudan, Eritrea, Ethiopia, west

Somalia, Namibia, Botswana, Zimbabwe, Mozambique, and north-eastern South Africa (Barlow et al., 2021; Mundy et al., 1992). The two subspecies differ in their feeding behaviour and morphology, which has highlighted the validity of subspecific status (Barlow et al., 2021). The morphological differences between the two subspecies are subtle, and delimitation relies on size differences (Barlow et al., 2021). *Necrosyrtes m. pileatus* is larger (wing length > 500mm) with a shorter stouter bill, while *N. m. monachus* is generally smaller (wing length < 500mm) with a longer, more slender bill (Barlow et al., 2021). A recent study by Barlow et al., (2021) using morphological measurement concluded that the size differences are due to geographic cline and not speciation, arguing that the species should be considered monotypic.

Hooded vultures are non-migratory with home range sizes that differ geographically, and on average, *N. m. pileatus* shows a more extensive monthly home range than *N. m. monachus* (Reading et al. 2019, Thompson et al. 2020). These scavengers frequently form flocks north of the equator in more urban areas and often feed at rubbish dumps, abattoirs, and sewage plants (Odino et al., 2014). In contrast, birds south of the equator are more solitary and found primarily in conservation areas (Odino et al., 2014; Ogada and Buij, 2011). There are regional differences in their movements too; vultures in The Gambia spend less time in urban areas than other West African populations, whereas birds tracked in Ethiopia (classified as *N.m. pileatus*) were seen moving into more urban areas (Reading et al., 2019; Thompson et al., 2020).

The first phylogenetic study conducted for New and Old-World vultures using genetic data was done by Wink (1995). This study has been a starting point for other studies regarding other vulture species' phylogeny and genetic variation. Since that study, no investigation into Hooded vulture phylogeny and taxonomy has been conducted, and several essential questions remain to be clarified. In particular, clarifying the taxonomic status of the subspecies is essential, as if the two subspecies are genetically distinct, then they should not be managed as one species, and current conservation policies must be updated.

Molecular markers used in vulture studies

The development of molecular technologies provides continuous opportunities to refine our understanding of genetic variation and diversity in natural populations. Even with the cost of full genome sequencing becoming feasible (Allendorf et al., 2010; Defaveri et al., 2013; Seeb et al., 2011; Zhang et al., 2011), other techniques, such as amplification of random repeats in genomic regions remain more accessible and widely used (Allendorf et al., 2010; Angeloni et al., 2012; Defaveri et al., 2013). A number of different molecular markers have been used to

trace the phylogeographic and phylogenetic histories of Old and New World vultures. These markers included mitochondrial markers such as the D-loop, cytochrome *b*, and COI regions, nuclear sequences, microsatellite markers, and nuclear protein coding loci (NPCL) such as RAG1, and allozymes (Arshad et al., 2009; Davidović et al., 2022; Ganbold et al., 2021; Lerner and Mindell, 2005; Mereu et al., 2019; van Wyk et al., 1992). The use of microsatellite markers due to their accessibility and high polymorphic nature has remained the choice of marker for studies resolving relationships below the species level (Defaveri et al., 2013; Morin et al., 2004; Narum et al., 2008; Payseur and Cutter, 2006; Väli et al., 2010).

Microsatellite markers, also known as simple sequence repeats, are simple short tandem nucleotide repeats scattered throughout the genome of all eukaryotes (Charlesworth et al., 1994; Defaveri et al., 2013; Fu et al., 2021; Messier et al., 1996; Powell et al., 1996; Valdes et al., 1993; Wenne, 2023; Wilson et al., 2021; Xu et al., 2022; Zhou et al., 2021). The number of repeats found varies among individuals from different species, making them useful in genetic studies focusing on relationships below the species level or among closely-related species (Messier et al., 1996). Microsatellite markers have been widely used to measure genetic diversity, population structure and connectivity, paternity and maternity, and information on genetic linkage maps (Wenne, 2023; Zhou et al., 2021). Microsatellite markers are among the few markers that can be implemented in cross-species and cross-genera analysis, with the bonus of reduced cost when developing these markers (Kpatènon et al., 2020; Pandey et al., 2021).

Microsatellites have been used on studies of vultures including Cape vulture (*Gyps coprotheres*), Bearded vultures (*Gypaetus barbatus*), Griffon vulture (*Gyps fulvus*), and in the Cinereous vulture (*Aegypius monachus*; Çakmak et al., 2019; Davidović et al., 2020; Kleinhans and Willows-Munro, 2019; Streicher et al., 2021). Kleinhans & Willows-Munro (2019) included a small sample of Hooded vultures in their microsatellite study focussing on Cape vultures. Streicher et al. (2021) amplified the same set of microsatellite loci for Bearded vultures. By amplifying the same loci in this study, we can directly compare the genetic diversity recorded in Hooded vulture to Cape vultures and Bearded vultures.

Analysis of microsatellite data: Inferring population structure and demographic history

Genetic diversity is the among the most important aspects to measure, when trying to conserve and better understand a species. The loss of genetic diversity occurs when populations experience bottleneck events, habitat fragmentation, and genetic drift, and when inbreeding

occurs in small populations (Hoban et al., 2023; Leigh et al., 2019; Söderquist et al., 2020). This loss of genetic diversity negatively affects the survival of a species by reducing its adaptive capability (Célia et al., 2012; Johnson et al., 2011; Söderquist et al., 2020). Thus, studying the change in effective population size is a key aspect of population genetics. Therefore, testing for these types of events occurring in a population is of utmost importance.

Inbreeding is another factor that impacts small and endangered populations. The effect of inbreeding occurs when the relatedness between individuals is increased and the number of reproductive individuals to select from are reduced.

There are a number of ways to infer population structure. The Program STRUCTURE implements a Bayesian clustering algorithm, that works by characterising population clusters based on the multilocus allele frequencies (Gilbert, 2016; Pritchard et al., 2000). The program uses a number of clusters (K) specified by the user to test the membership probability for each individual, by using Markov chain Monte Carlo (MCMC) simulations (Gilbert, 2016; Pritchard et al., 2000). These MCMC simulations also assume Hardy-Weinberg and also linkage equilibrium within the groups (Gilbert, 2016; Pritchard et al., 2000). This type of analysis helps to visualize how individuals are proportionally assigned over a landscape (Gilbert, 2016). Assessing which K is best-fit for a specific system can be done using two main post hoc tests. The Evanno method, which infers the ΔK by assuming that the best-fitting K is where the biggest change in magnitude for the second order rate of change in $P_r(X|K)$ against the successive K values occurs (Evanno et al., 2005; Gilbert, 2016). The second method is known as the Puechmaille method, which infers the best-fitting K by calculating the MedMeaK, MaxMeaK, MedMedK and MaxMedK (Gilbert, 2016; Puechmaille, 2016). This method calculated the best-fitting K by inferring the cluster at a given K value based on the mean or median of their individual's membership coefficients (Gilbert, 2016; Puechmaille, 2016).

Approximate Bayesian computation (ABC) was an idea that dates back to the 1980s, when Donald Rubin discussed the interpretation of Bayesian statements and then described a hypothetical sampling mechanism that yields a sample from the posterior distribution (Marin et al., 2012; Sunnåker et al., 2013). The first time it was used in population genetics was by Tavaré et al. (1997), who introduced the ABC method as a rejection technique, allowing for bypassing the likelihood computation by simulating the corresponding distribution (Marin et al., 2012; Tavaré et al., 1997).

However, this version of ABC could only be implemented in a few cases (Marin et al., 2012). The first time the complete ABC algorithm was implemented was by Pritchard et al. (1999); they extended the algorithm implemented by Tavaré et al. (1997) to allow for continuous sampling of solution space. This basic idea of ABC is based on the notion that; using summary statistics of a population, coupled with a low tolerance rate, should produce an acceptable approximate for posterior distribution (Marin et al., 2012; Pritchard et al., 1999). The use of ABC in population genetics was established by Beaumont et al. (2002) when the methodology was extended, and its suitability for use in population genetics was discussed (Beaumont et al., 2002; Marin et al., 2012). This algorithm was then modified by Marjoram et al. (2003) to impellent Markov Chain Monte Carlo (MCMC), which removed the need for the calculation of likelihood satisfying ABC requirements (Marin et al., 2012; Marjoram et al., 2003). Wilkinson (2008) suggested a change in panoche, which replaces the error rate resulting from the loose acceptance conditions in the likelihood-free sampler with an exact inference-controlled approximation for the target (Marin et al., 2012; Wilkinson, 2008). This essentially resulted in a convolution of the target with a kernel function (Marin et al., 2012; Wilkinson, 2008).

ABC is popular because Bayesian inference about populations can be calculated without the need for likelihood models (Beaumont et al. 2002, Marjoram et al. 2003, Wilkinson 2008, Marin et al. 2012). This algorithm can thus implement implicit computer models that generate a sample data set instead of returning likelihoods that are compared to the collection of summary statistics (Beaumont et al., 2002; Wilkinson, 2008). This analysis method has been implemented in different disciplines of genetics. This algorithm has been used to compare the evolutionary dynamics of protein networks, calculate the divergence of two subspecies of the desert locust (*Schistocerca gregaria*), and contrast the evolutionary history and genetic contact in the northern and southern White rhinoceros (*Ceratotherium simum;* Chapuis et al., 2020; Moodley et al., 2018; Ratmann et al., 2007). Therefore, the use of ABC modelling in comparing changes in population demographics to better understand the changes observed in populations is highly viable.

Aims of the MSc study:

This study aims to clarify the taxonomic status of the two Hooded vulture subspecies and examine the genetic diversity of the species across its pan-African distribution using a set of microsatellite loci (Chapter 2). A second aim is to assess the overall genetic health and diversity

of the edge population found in South Africa and do a comparison with a population located in the centre of the species distribution (Chapter 3). This study also aims to provide important insight into the population dynamics of Hooded vultures in South Africa and will examine processes such as nest use, dispersal and natal philopatry of the southern African population found in the greater Lowveld area of South Africa (Chapter 4).

Each chapter of this thesis is written as a manuscript. As such there may be some repetition in key information. Data generated in this study will be used to improve the species' current conservation and will significantly enhance our understanding of the species.

Chapter 2

Revisiting the taxonomy of Hooded vulture subspecies *Necrosyrtes monachus monachus* and *Necrosyrtes monachus pileatus*

Rynhardt Le Roux, Lindy J. Thompson, Bettine van Vuuren and Sandi Willows-Munro

Abstract

Hooded vultures (*Necrosyrtes monachus*, Temminck, 1823) are widely distributed in the savannahs and grasslands of Africa. This species is divided into two subspecies: *Necrosyrtes monachus monachus* (Temminck, 1823) occurs in northwest Africa, while *Necrosyrtes monachus pileatus* (Burchell 1824) is found in southeast Africa. *Necrosyrtes monachus. monachus* occurs at high densities and in urban areas. In contrast, *N. m. pileatus* occurs at lower densities, and is almost exclusively limited to conservation areas. In this study, we aimed to examine the phylogeographic structure of the Hooded vulture by including samples (n = 42) from across the species' African distribution (six countries) using a panel of thirteen microsatellite markers. In particular, the aim is to clarify the taxonomic status of the two subspecies using classic frequency-based phylogeographic analyses and Approximate Bayesian computation (ABC). STRUCTURE analyses did not support the genetic differentiation of the two subspecies, instead recovering five genetic clusters (samples collected from Senegal, Ghana, Ethiopia, and South Africa were all genetically different, while samples from Ghana, Uganda and Angola were clustered together). AMOVA recovered only 11.19% (p–value = 0.000) variance between the two proposed subspecies. The contemporary gene flow between the subspecies was 18.8%, indicating gene flow between the two subspecies is not restricted and that 18.8% of genes are shared between the subspecies per generation. The genetic data suggest that the differentiation seen is due to isolation-by-distance. This is supported by a positive correlation between genetic distance and geographical distance recovered by a Mantel test ($r = 0.244$, $R^2 = 0.0594$ and p-value = 0.0001). Approximate Bayesian computation (ABC) analyses were used to examine the magnitude, timing and direction of gene flow between the two subspecies and also the timing of bottlenecks and expansions. Simulations indicated bidirectional gene flow between the two subspecies occurred approximately 51000 - 40000 years ago (p-value = 0.220). The ABC analysis also indicated that *N.m. pileatus* experienced an ancient bottleneck event (p-value = 0.720) around 77000 years ago, while *N. m. monachus* experienced a more recent bottleneck event (p-value = 1.000) around 8000 years ago. The timing of these events corresponds to local shifts in

climate, which coincided with a decrease in temperature and an increase in forest coverage. This study thus finds no evidence to support the current subspecies classification, as the two subspecies do not form cohesive evolutionary lineages. Instead, this study supports claim made by Mundy in that the morphological, behavioural, and ecological differentiation seen is due to adaptation to local environments and not speciation. This study further indicated that the species experiences different demographical changes. Given the ecological and behavioural differences seen at the extremes of their distribution, Hooded vultures may benefit from more localised management programs.

Key words: Hooded Vulture, subspecies classification, Africa, Taxonomy, spesiation

Introduction

Hooded vultures (*Necrosyrtes monachus*, Temminck, 1823) are widely distributed across Africa, south of the Sahara, except in heavily forested regions around the equator. Like other African vulture species, Hooded vulture numbers have declined rapidly in recent years (Ogada et al., 2016; Ogada and Buij, 2011; Teklemariam and Afework, 2021). Population declines have been linked to various anthropogenic factors, which include intentional and accidental poisoning, collisions with and electrocutions on electrical infrastructure and the trade of body parts for use in traditional medicine (Boakye et al., 2019; Henriques et al., 2020; Mashele et al., 2021; Odino et al., 2014; Owolabi et al., 2021; Saidu and Buij, 2018; Williams et al., 2021). The International Union for Conservation of Nature's Red List of Threatened Species lists the Hooded vulture as Critically Endangered (BirdLife International, 2022). Sustainable conservation interventions are needed for the species; however, these are extremely difficult to design and implement for continentally distributed species (Thornton et al., 2018) such as the Hooded vulture.

In Hooded vultures, two subspecies are recognised (Figure 2.1). *Necrosyrtes monachus monachus* (Temminck, 1823) is found in Northwest Africa, and *Necrosyrtes monachus pileatus* (Burchell 1824) occurs in East and Southern Africa (Mundy et al., 1992; Mundy, 2021; Teklemariam and Afework, 2021).

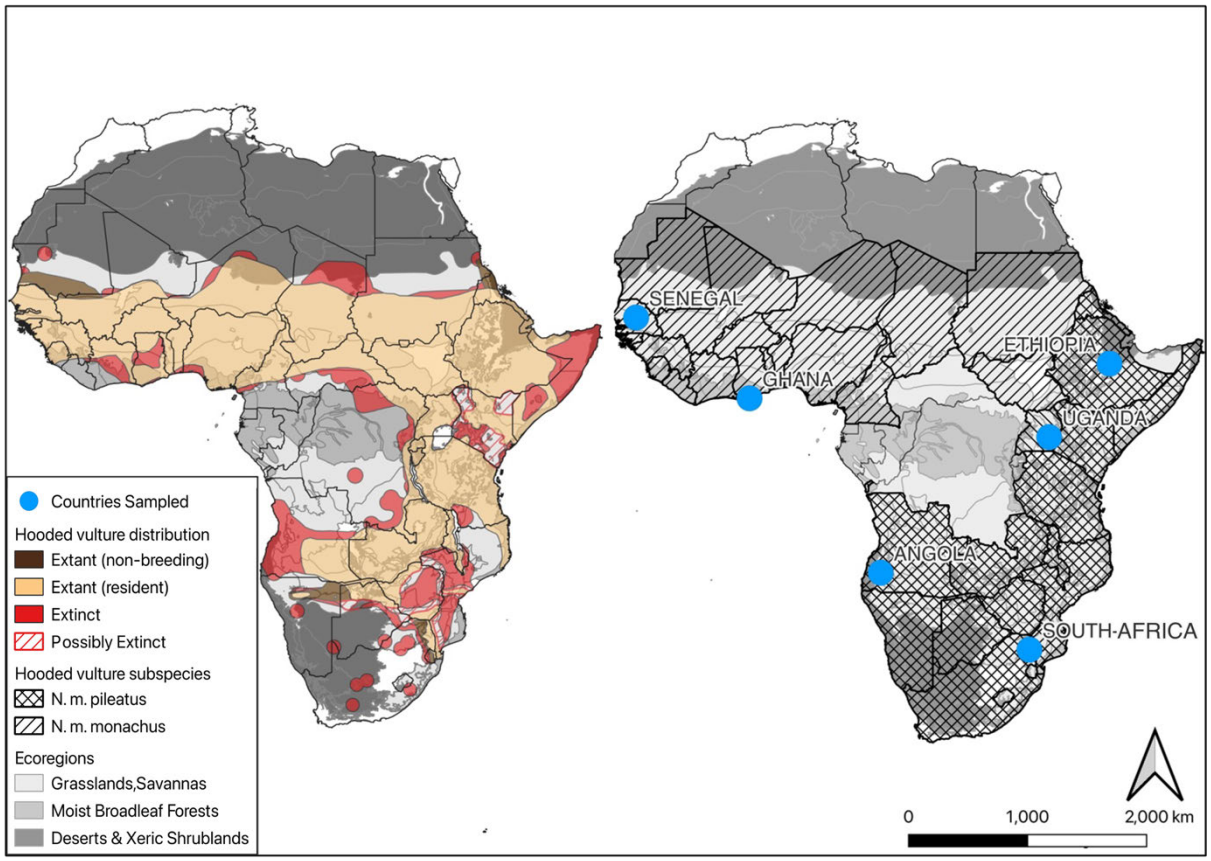

Figure 2.1: Distribution of the Hooded vulture (*Necrosyrtes monachus*) across its pan-African distribution (left). The distribution of the two subspecies: *Necrosyrtes monachus monachus* indicated by the diagonal checkers pattern and *Necrosyrtes monachus pileatus* indicated by the diagonal lines (right). The distribution of the ecoregions (Savannas and Grassland, Moist, Broadleaf forests, and Deserts) are also included as the distribution of the species is associated with ecoregions.

Although these two subspecies differ in body size, level of commensalism (amount of time spent in urban vs non-urban areas), home-range sizes and populations of the two subspecies occur in different densities, there is still debate on the taxonomic status of the two subspecies (Barlow et al., 2021; Donsker and Rasmussen, 2022; McLachlan and Liversidge, 2016; Mundy, 2021). For example, the size difference could result from a west-to-east size cline rather than divergence (Mundy 2021). Similarly, a study that examined cranial biometrics found no significant difference in the biometric measurements between the two subspecies (Barlow et al. 2021), suggesting that the two subspecies may not be biologically relevant. In contrast, telemetry studies have shown that *N. m. pileatus* have more extensive home ranges than *N. m. monachus* (Thompson et al. 2020). This disparity may, in part, be due to the species' flexibility in feeding strategies, with *N. m. monachus* spending a larger proportion of time in urban areas

and less time searching for food (Barlow and Fulford, 2013; Gbogbo and Awotwe-Pratt, 2008; Mundy et al., 1992), while *N. m. pileatus* occurs in more natural areas which require larger home ranges for feeding (Teklemariam and Afework, 2021). *Necrosyrtes monachus monachus* could be considered an urban exploiter, foraging from dumps, garbage cans and abattoirs, while *N. m. pileatus* actively avoids urban areas (Gbogbo et al., 2016; Gbogbo and Awotwe-Pratt, 2008; Mullié et al., 2017). In The Gambia, *N. m. monachus* has been observed to regularly scavenge along beaches and ingest marine carrion, whereas *N. m. pileatus* exclusively exploits savanna habitats (including supplementary feeding sites for vultures in northeast South Africa) (Barlow et al., 2022; Mundy et al., 1992; Reading et al., 2019). Local adaptation is not unexpected in species with such wide distributions. Given enough time, local adaptation could drive the divergence of populations toward distinct fitness optima in heterogeneous environments (Kawecki and Ebert, 2004; Nosil and Feder, 2012; Rundle and Nosil, 2005). This process could occur more rapidly if gene flow between populations becomes limited (Bard, 2022; Clark et al., 2022; Kessler et al., 2022; Wang and Coop, 2022). In the case of species with large geographical distributions, where populations have adapted to different ecological environments, these adaptations can be maintained, and they can still be considered subspecies as long as gene flow between the subspecies persists. However, when this gene flow is interrupted by any geographical or reproductive barrier, it could ultimately lead to speciation (Butlin et al., 2014; Clark et al., 2022; Pinho and Hey, 2010; Yeaman and Otto, 2011).

In the Hooded vulture, it can be hypothesised that changes in the distribution of the forests and deserts of central and north Africa could impact and reduce gene flow between the populations of Hooded vultures in West and East Africa, leading to the evolution of the two subspecies. As these changes in the habitat act as geographical barriers to reduce geneflow between the populations. During the middle to late Pleistocene, the Sahara consisted mainly of grasslands suitable for Hooded vultures, while today, this area is mostly uninhabitable (Ehrmann et al., 2017; Hoag and Svenning, 2017). In addition, the moist forests of central Africa have experienced both expansion and contraction events, which could have impacted Hooded vulture distribution. Expansion in the distribution of forests is also linked to the pre-Holocene African humid periods. During these periods, the landscape consisted of more lakes and denser vegetation cover (Lebamba et al. 1998, Ehrmann et al. 2017, Hoag & Svenning 2017), a habitat unsuitable for foraging for Hooded vultures. These climate-driven changes have been hypothesised to be responsible for the phylogeographic structure observed in other savannah and grassland species such as rhino (*Ceratotherium simum*) and lion (*Panthera leo*) (Cooper et

al., 2021; Moodley et al., 2018). It seems likely that similar processes have led to the phylogeographic structure in the Hooded vulture.

There has been considerable debate over the validity and usefulness of delimiting subspecies (Haig et al., 2006; Mallet et al., 2004). However, identifying groups of populations that are distinct is an important consideration in the conservation and maintenance of genetic and biological diversity. Subspecies are defined as being geographically and genetically distinct but still able to interbreed (Haig et al., 2006; Mayr, 1953). In cases where genetic data are used to justify subspecies delimitation, a subspecies can be defined as a population or collection of populations that represent separately evolving evolutionary lineages. The divergence between subspecies could result from geographical isolation, ecological specialisation, or any other forces that could restrict gene flow such that subspecies become genetically distinct (Taylor et al. 2016).

Africa has experienced it fair share of climactic changes that has caused a shift in vegetation leading to geographical isolation in various species. The biggest changes have been observed in the northern parts of Africa during a humid period in the climate that resulted in a greener Sahar dessert (Lebamba et al. 1998, Ehrmann et al. 2017, Hoag & Svenning 2017),. This climatic event occurred around the early Holocene (Lebamba et al. 1998, Ehrmann et al. 2017, Hoag & Svenning 2017), During this period the African climate was more humid and various lakes could be observed in the Sahara Desert in the low laying areas (Ehrmann et al., 2017; Hoag and Svenning, 2017) . During the Last Glacial Maxima is also where a great shift in the African climate and vegetation is observed, as during this time the climate was cooler than the present-day climate (Ehrmann et al., 2017; Hoag and Svenning, 2017). During this period a decrease in savanna habitat and sea levels was observed and an increase in the central moist forest occurred (Ehrmann et al., 2017; Hoag and Svenning, 2017).

This study aims to use microsatellite data to either support or refute the current subspecies delimitation. We first test for the presence of population structure across the distribution of the species and test if the two subspecies are genetically distinct and represent different evolutionary lineages. Second, we determine the timing, magnitude and direction of gene flow between the two subspecies. Finally, we use Approximate Bayesian computation (ABC) simulations to determine if the two subspecies have undergone different demographic changes linked to local environmental conditions. If the two subspecies are ecologically and genetically

distinct, it may require that each subspecies be managed separately. Alternatively, if the two subspecies are not genetically distinct then the current subspecies delimitation may be invalid.

Materials and methods

Sampling

Samples from a total of 42 Hooded vultures were used for this study. *Necrosyrtes monachus monachus* (Figure 2.1) was represented by ten samples from Senegal and ten from Ghana. *Necrosyrtes monachus pileatus* (Figure 2.1) included samples from Ethiopia (N = 9), Uganda (N = 1), Angola (N = 1) and South Africa (N = 11) (Appendix 2.1).

DNA extraction and microsatellite amplification

DNA was extracted from feather, blood and archival toepad samples using the NucleoSpin® Tissue kit (Macherey-Nagel, Germany). Minor modifications to the extraction protocol were made to ensure sufficient DNA yield from feather and archival toepad samples. The modifications included extending the incubation times (at 56°C) of samples in lysis buffer with proteinase K to 24 hours for blood and feather samples and 48 hours for toepad samples. The lysate was then incubated in B3 buffer for 45 minutes (70°C), the final volume of pre-warmed Buffer BE was added to the spin column and centrifuged and reheated after the first centrifuge for 5 minutes, there after reapplied to the membrane for a final centrifuge step.

A microsatellite panel consisting of 13 loci amplified in five multiplex reactions was selected for this study (Table 2.1). These loci have been used previously to assess the below-species genetic diversity of other Old-World vulture species, including Cape vultures (*Gyps coprotheres*) and Bearded vultures (*Gypaetus barbatus*; Kleinhans and Willows-Munro, 2019; Streicher et al., 2021). Each 10 µl reaction consisted of ~2-30 ng of DNA template, 5 µl of Multiplex TEMPase mix (AMPLIQON, Denmark), 0.2 µl of 0.2 µM primers and purified water. Thermocycler parameters were as follows: initial denature at 94°C for 15 min, followed by 94°C for denaturing, 60°C for annealing and then 72°C for elongation for a total of 35 times. Amplified products were sent to the Central Analytic Facility (CAF) at Stellenbosch University, South Africa, for fragment analyses using a ABI3730xl machine. The genotype scoring was done using GeneMarker v2.6.3 (Soft Genetics). All multiplex reactions were reamplified up to five times and cross-referenced to ensure data reliability.

Table 2.1: List of microsatellite loci used in genetic analysis of Hooded vulture (*Necrosyrtes monachus*). Primers used for amplification, fluorescent dyes used for tagging the forward

primers, annealing temperature (Tm) used in each reaction for the amplification of specific microsatellite loci with the combination of loci amplified together in multiplex reactions.

Locus	Primer pair	Dye	Tm	Motif	Multiplex
BV2	F: CAGCATGTTATTTTGGCTGC	HEX	60	CA_{11}	A
	R: TTGCTAAACCGGTTAGAAGTTG				
BV6	F: AATCTGCATCCCAGTTCTGC	HEX	60	CA_{11}	D
	R: CCGGAGACTCTCAGAACTTAAC				
BV8	F: TGGCATGCTGCTATGAGAAC	FAM	60	CA_{11}	C
	R: GTGCTTTGCATGCTTTTACTC				
BV9	F: ATCTAGGGACATCGAGGAGC	HEX	60	TA_6 CA_{11}	D
	R: ACAGGGATGCAGGTAAGCC				
BV11	F: TGTTTGCAAGCTGGAGACC	HEX	60	CA_{22}	B
	R: AAAAGCCTTGGGGTAAGCAC				
BV12	F: TCAGGTTTTGACGACCTTCC	FAM	60	CA_{15}	C
	R: GTGGTAACGGAGGAACAAGC				
BV13	F:AAAACAGAGTTTTCACATTTTCATAAG	FAM	60	CA_{16}	A
	R:TTCAGGAAACAGAAGCATGAAC				
BV17	F: TGATGTGCAGATGCGTGAC	HEX	60	CA_{11}	C
	R: GGACTCTGATGAAGCCAAGC				
BV20	F: GAACAGCACTGAACGTGAGC	HEX	58	CA_{13}	E
	R:GTTTCTCCTGACAGTGAAATAACTC				
Gf3H3	F: GTAGAATAATTTGCTCCTGG	FAM	60	CT_{12}	D
	R: GTGAAGGCACCTCATAGACA				
Gf8G	F: TGAGCAGGTGAGTCCAGAAG	FAM	60	CT_8C TC_2	B
	R: GCTCTCCTGTCATCTTGCAT				

Gf9C	F: GGTGGACATTACATACACTG R: CAAGGAATCTGGACTACTAA	HEX	60	TC_{10}+CT_9 C CA_5T AC_4	A
Gf11A4	F: GATCCCTTCCAACCGAAAAT R: TGGTGACCAACGGAAGTGTG	HEX	60	$CTCTT_{17}$	C

Estimating genetic diversity

Cervus v3.0.7 (Kalinowski et al., 2007) was used to estimate the polymorphic information content (PIC) of each locus, and to ensure no duplicates were included. Genepop v4.2 (Hubisz et al., 2009) was used to test for deviation from Hardy-Weinberg equilibrium (HWE). Genetic diversity within each subspecies and across the species as a whole was estimated by calculating the number of alleles, observed heterozygosity, and unbiased expected heterozygosity using GenALEx v6.502 (Peakall and Smouse, 2006).

Phylogeographic structure of Hooded vulture

Bayesian assignment tests were performed in STRUCTURE v2.3.4 (Hubisz et al., 2009) using the admixture ancestry model with correlated allele frequencies. LOCPRIOR was also implemented, with geographic locality used as prior. Ten independent runs were performed, each consisting of 1 000 000 Markov chain Monte Carlo (MCMC) replicates with a burn-in of 100 000 and a proposed number of genetic clusters (K) ranging from 1 to 10. The optimal number of genetic clusters was estimated using STRUCTURE Selector, implementing the Puechmaille method (Li and Liu, 2017; Puechmaille, 2016). The Puechmaille method is best when sampling is unequal (Gilbert, 2016) as is the case here where some localities are represented by single samples. The membership probabilities (Q-values) for each genetic cluster and individual were estimated using STRUCTURE Harvester (Earl and vonHoldt, 2012). Pophelper (Francis, 2017) was used to visualise the STRUCTURE bar plots.

A Principal Coordinate Analysis (PCoA) was performed in GenAlEx v6.502 (Peakall and Smouse, 2006). The genetic differentiation of the two subspecies was tested using an Analysis of Molecular Variance (AMOVA). The AMOVA was performed in Arlequin v3.5.5.2 (Excoffier and Lischer, 2010). The genetic relationships among both individuals and populations of Hooded vultures were also visualised using EDENetworks v2.18 (Kivelä et al., 2015). This program plots the individuals or populations as nodes in a network graph with

connections between nodes weighted by their pairwise genetic distance (Fst). In this analysis, populations included individuals grouped by collection locality. The allele sharing and Manhattan linear protocols were selected to construct networks.

A Mantel test (Mantel 1967) was performed in GenALEx v6.502 (Peakall and Smouse, 2006) to assess if there was a correlation between genetic distance and geographic distance. The Mantel test was performed for 9 999 permutations, using Nei's genetic distance and geographic distance (km).

Gene flow between *N. monachus monachus* and *N. monachus pileatus*

Gene flow between the subspecies was estimated using BayesAss v1.3 (Wilson and Rannala, 2003) and ABC simulations. BayesAss analyses consisted of 10 000 000 iterations, with a burn-in of 1 000 000 and leaving a total of 90 000 iterations for downstream analysis. Initial runs were performed to ensure MCMC chain convergence, and delta values were adjusted to achieve a 20-60% acceptance rate (Wilson and Rannala, 2003). The final delta values were allele frequency = 0.30, delta migration rate = 0.10 and delta inbreeding coefficient = 0.50. We consider a migration rate lower than 0.100 or 10% to indicate genetic isolation, as this has been seen to indicate isolation in other vulture species (Hastings, 1993 Kleinhans and Willows-Munro, 2019; Streicher et al., 2021).The timing, direction, and magnitude of gene flow were also incorporated into ABC simulations (see below).

Modelling demographical changes in each subspecies using Approximate Bayesian Computation (ABC Toolbox)

Determining the timing and extent of gene flow between closely related taxa is difficult. This is particularly true where there is no obvious barrier to restrict gene flow, and individual taxa may not have evolved in isolation. In most cases, species barriers are established progressively, depending on the size and degree of reproductive isolation of the species, and genetic variation is shared over periods that are longer than previously thought (Brandvain et al., 2014; Edelman et al., 2019; Novikova et al., 2016). Demographic changes such as bottlenecks or rapid population expansion can also significantly affect the genetic diversity in populations (Collier et al., 2010; Heinrichs et al., 2016).

In this study, we use ABCToolbox v. 1.1 (Wegmann and Excoffier 2010) to model different demographical events and test several complex scenarios to determine 1) when and to what

extent gene flow has occurred between the two subspecies of Hooded vultures and 2) if and when the two subspecies experienced a significant reduction or increase in population size.

Three sets of models were tested. The first set (Set A, Figure 2.2) of simulations was conducted on each subspecies individually and was used to test if subspecies had experienced a significant expansion (current population size N_0 is larger than ancestral population size N_1 over a period of time T; Scenario 2 & 4), reductions (current population size N_0 is smaller than ancestral population size N_1 over a period of time T; Scenario 3 & 5) or series of bottlenecks (ancestral population (N_1) experienced a bottleneck at time (T_1), stabilised to a population size N_{New} , and then N_{New} experienced a second bottleneck at a later time ((T_2) Scenario 6). A null model where the ancestral population size (N_1) is the same as the current population size (N_0) over a period of time (T) was also included. These models were simulated using different time (T) of expansion or bottleneck: ancient expansion with T_1 prior from 1 and 125 000 years ago (Scenario 2) versus recent expansion with T_2 from 1 year to 12 500 years ago (Scenario 4), ancient bottleneck (Scenario 3), versus recent bottleneck (Scenario 5). The T prior distribution was selected based on changes in the climate that led to changes in forest cover across Africa. As a savannah species, Hooded vulture distribution is expected to change in response to the contraction or expansion of central African forests. The central African forest experienced significant climate-linked changes after the last glacial maximum (Adams and Faure, 1997; Elenga et al., 1994).

Set B included three models to estimate magnitude and direction (unidirectional or bidirectional) of gene flow between the two subspecies post divergence where both subspecies *N. m. monachus* ($N_{0\_N.m.\ monachus}$) and *N. m. pileatus* ($N_{0\_N.m.\ pileatus}$) experienced population decreases. In this case $T_{post\text{-}divergence}$ was set based on results from Set A simulations. The magnitude and direction of gene flow were then tested at different times ($T_{time\ of\ gene\ flow}$). This was to investigate how changes in the landscape would affect gene flow between the two subspecies. The first three sets tested the same time frame as to when the bottleneck events occurred for the two subspecies ($T_{time\ of\ gene\ flow}$ = 7 709- 77 064 years ago, Scenarios 7-9). The second set of three scenarios was set ($T_{time\ of\ gene\ flow}$) during the last glacial period (LGP) during the late Pleistocene (14 000-106 000 years ago, scenarios 16-18). The next three scenarios set $T_{time\ of\ gene\ flow}$ during the last glacial maximum (LGM) (14 000-26 000 years ago, Scenarios 10-12). Early gene flow was tested in the final three scenarios during the last glacial period (26 000-106 000 years ago, Scenarios 13-15).

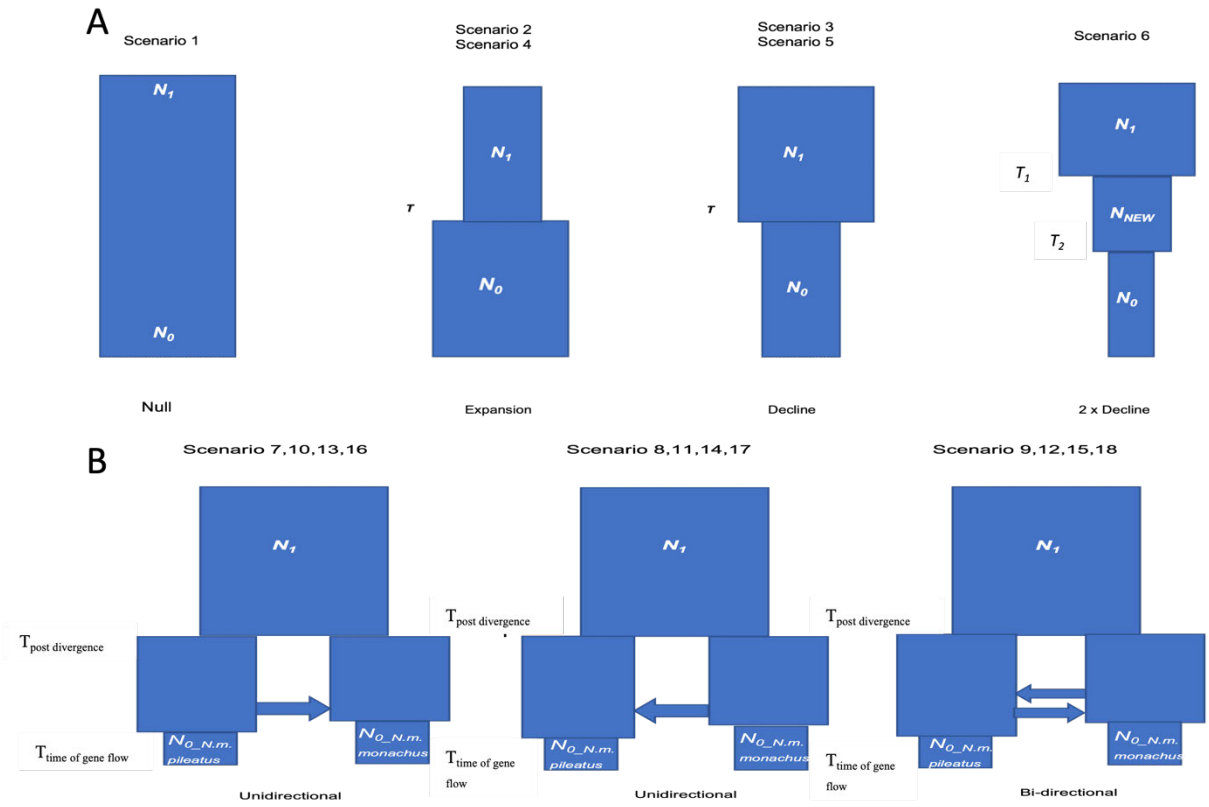

Figure 2.2 Three sets of models used to estimate demographic changes in Hooded vulture subspecies *N. m. monachus* and *N. m. pileatus.*

In total 18 different scenario were run. These scenarios were simulated for 200 000 iterations and 5 000 iterations were used for the estimation of posterior probability and parameter estimations. The actual time of these events, was converted from generational time,13 years (BirdLife International, 2022).

Results

Thirteen microsatellite markers were successfully amplified in all 42 Hooded vulture samples. Missing data across loci included in the final dataset varied, however remained minimal (mean = 3.48%). No duplicates were detected when running identification test and all samples were included in further analysis. The mean frequency of null alleles (N_o) for the Hooded vulture across Africa was 13% (Appendix 2.2). Eight loci (BfV2, BV13, Gf8G, BV8, BV 12, BV17, BV9, Gf11A4) showed elevated levels of null alleles (Appendix 2.2): However, these elevated levels of null alleles will not affect the overall population genetic structure as F_{ST} did not indicate a significant difference between the correlated and uncorrelated F_{ST} (p-value > 0.05). The number of alleles per locus ranged from 5.000 – 14.000 (Appendix 2.1). Nine loci (BV 13,

Gf9C, BV 11, Gf8G, BV 12, Gf11A4, BV 6, Gf3H3, BV 20) were identified to be moderate to highly informative (PIC > 0.5). PIC values of other loci ranged from 0.367 to 0.470.

The observed heterozygosity for both subspecies (H_o) was similar (*N. m. monachus* H_o = 0.485 and *N. m. pileatus* H_o = 0.424) (Table 2.2, Appendix 2.2). *Necrosyrtes m. monachus* showed higher levels of effective alleles (N_e = 3.920) compared to *Necrosyrtes monachus pileatus* (N_e = 2.540) (Table 2.2).

Table 2.2: Genetic diversity estimates of Hooded vulture *Necrosyrtes moachus* and *Necrosyrtes monachus monachus* and *Necrosyrtes monachus pileatus*. The mean number of alleles (A), the mean number of effective alleles (Ne), the mean null allele frequency (N_o), the mean Shannon's information index (I), the mean observed heterozygosity (H_o), the mean expected heterozygosity (H_e), the mean unbiased expected heterozygosity (uHe), the mean deviation form Hardy Weinberg Equilibrium (HWE), mean inbreeding coefficient (F).

Species	**A**	**Ne**	**N_0**	**I**	**H_o**	**H_e**	**uHe**	**HWE**	**F**
Necrosyrtes monachus	9.000	3.417	0.131	1.452	0.451	0.643	0.652	0.000	0.333
N. m. monachus	7.154	3.920	0.142	1.490	0.485	0.687	0.705	0.000	0.337
N. m. pileatus	5.231	2.540	0.144	1.052	0.424	0.519	0.532	0.000	0.165

Phylogeographic structure of Hooded vulture

The Puechmaille method recovered five genetic clusters as optimal (Figure 2.3). The two subspecies do not cluster together (Q = 0.206, *N.m. monachus*) and (Q = 0.200, *N. m. pileatus*); rather, genetic similarity seems to be geographically correlated. Individuals collected from the same localities had high membership coefficient values (Q = 0.928 Senegal; Q = 0.718 Ghana; Q = 0.800 Ethiopia; Q = 0.945 South Africa).

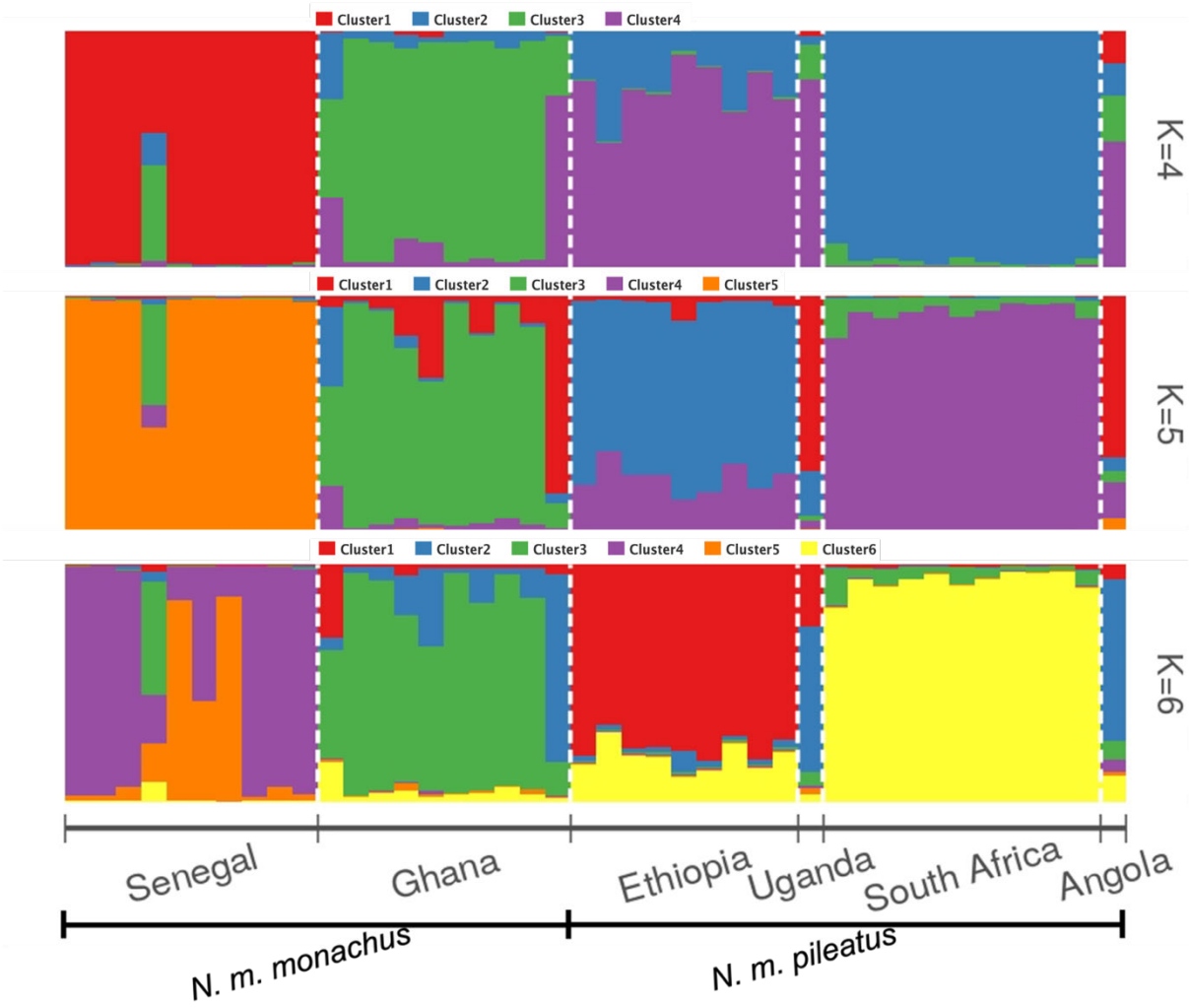

Figure 2.3: The STRUCTURE bar plot for the Hooded vulture (*Necrosyrtes monachus*). The optimal number of genetic clusters (K) was estimated as five by the Puechmaille method.

The PCoA also did not cluster the members of each subspecies together. Instead, individuals collected from the same locality are clustered together and associations between localities representing an east-to-west cline.

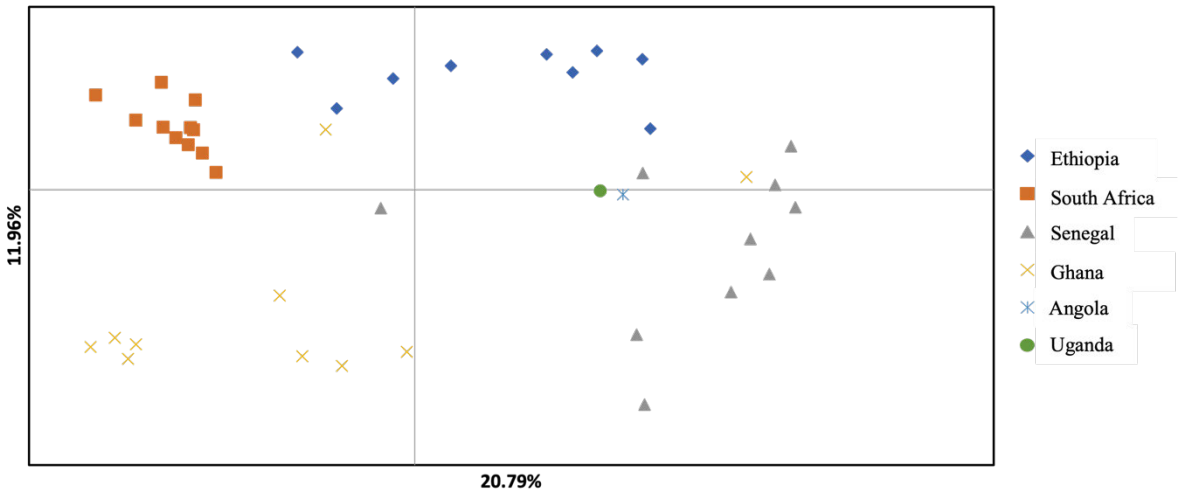

Figure 2.4: Principal Coordinate Analysis for Hooded vulture (*Necrosyrtes monachus*). Plotting individuals together based on shared genetic alleles.

The AMOVA supported the results from STRUCTURE and PCoA and recovered only 11.19% variance between the two subspecies, and 18.88% within the populations from each subspecies sampled (Table 2.3). Most variation was between individuals (69.93%). AMOVA test indicated a (p – value = 0.000).

Table 2.3: AMOVA grouping individuals into the two subspecies of Hooded vulture (*Necrosyrtes monachus monachus* and *Necrosyrtes monachus pileatus*), indicating the (%) variation among the subspecies and within the populations sampled (p-value = 0.000).

Source	Df	Sum of square	Variance component	% Variation	Fixation Indices
Among subspecies	1	22.681	0.440	11.19	FIS: 0.213
Within populations	40	169.391	0.742	18.88	FST: 0.112
Within individuals	42	115.500	2.750	69.93	FIT: 0.301
Total	83	307.571	3.933	-	p-value = 0.000

The network constructed using EDENetworks similarly did not find evidence for a genetic discontinuity between the two subspecies, rather that individuals from *N. m. monachus* share alleles with individuals from *N. m. pileatus* (Figure 2.5 A). The size of the nodes also indicates the strength of these similarities shared. Ethiopia is the stepping-stone population as it shares genetic similarities with Ghana and Senegal (Figure 2.5 B).

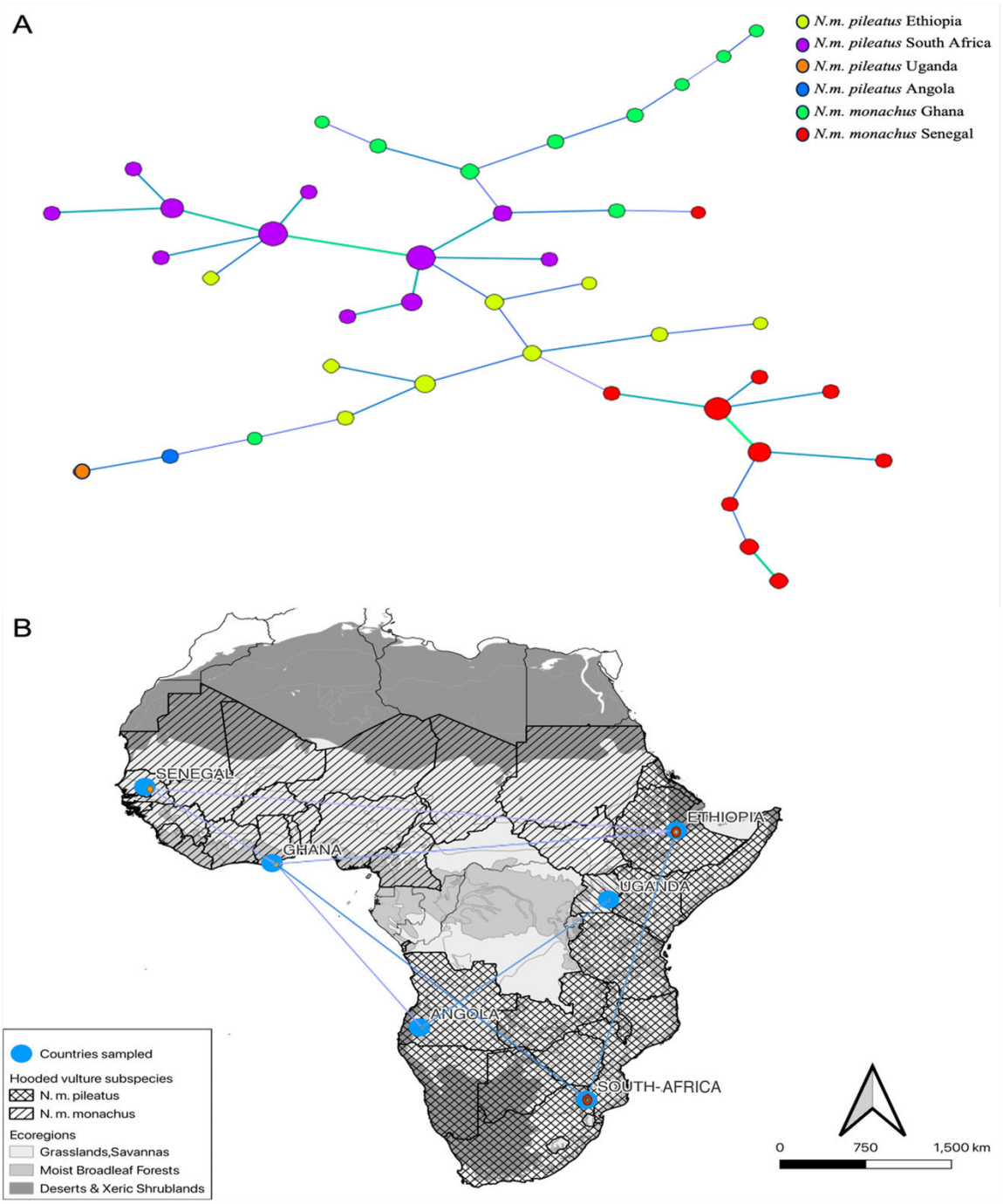

Figure 2.5: Network constructed in EDENetworks linking individuals of Hooded vulture (*Necrosyrtes monachus)* by the strength of genetic correlation between each individual (A), and sampling localities (B).

The Mantel test shows a positive correlation (r = 0.244, R^2 = 0.0594, and p-value = 0.0001) between genetic and geographic distance. This supports isolation-by-distance as main driver of genetic difference rather than divergence.

Contemporary gene flow between *N. monachus monachus* and N. *monachus pileatus*

Using BayesAss the rate of migration between *N. m. pileatus* (source) and *N. m. monachus* (sink) is only 0.015. When *N. m. monachus* (source) and *N. m. pileatus* (sink) is 0.188. The latter migration rate is above the threshold of 0.100 or 10% (Hastings, 1993) and suggests that there is no barrier to gene flow between the two subspecies.

Table 2.4: Migration rates estimated using BayesAss between the two subspecies of Hooded vulture (*Necrosyrtes monachus*). The gene flow has been tested both ways, one subspecies being the source and the other the recipient of the genetic flow.

Source Recipient	*N. m. monachus*	*N. m. pileatus*
N. m. monachus	0.812	0.015
N. m. pileatus	0.188	0.986

Modelling demographical changes in each subspecies using Approximate Bayesian Computation (ABC Toolbox)

For most of the ABC simulations the probability of the specific event occurring does not differ with more than 3 Bayes Factors. Thus, these results should be interpreted with caution, as a clear distinction to which event occurred cannot be made.

In the first set of scenarios examining each subspecies individually the most probable scenario for *N. m. pileatus* included an ancient bottleneck event (Table 2.5 and Appendix 2.5) around 77 066.99 years ago (p-value = 0.720). The most probable scenario for *N. m. monachus,* was a stable ancestral population (p-value = 1.000) undergoing a recent bottleneck event approximately 7 703 years ago (p-value = 1.000) (Table 2.5 and Appendix 2.6).

In the second set of simulations testing the timing and direction of gene flow between the two subspecies, the most probable scenario (Scenario 9) was bidirectional gene flow between the two subspecies (Table 2.6 and Appendix 2.4) around 51 143 – 40 625 years ago (p-value = 0.220).

Table 2.5: Approximate Bayesian computation (ABC) posterior estimations for six demographic scenarios for the Hooded vulture (*Necrosyrtes monachus*) subspecies (*N. m. monachus* and *N. m. pileatus*). These demographical scenarios were a stable null hypothesis, an ancestral expiation, an ancestral bottleneck, a more recent bottleneck and expiation, and then finally, two bottleneck events, a recent and ancient event.

Population	Scenario	Description	*NANC	*NCUR	Time (yrs[#]) LB	Time (yrs[#]) Mode	Time (yrs[#]) UB	Marginal density	p-value
Necrosyrtes monachus pileatus	1	Stable	459 597	36 364	9 918	75 760	115 756	0.014	0.630
	2	Ancient expansion	28 485	85 136	1 366	26 616	110 384	4.468×10^{-15}	0.000
	3	Ancient bottleneck	555 777	39 397	10 517	77 067	115 887	0.016	0.720
	4	Recent expansion	80 808	160 762	748	3 543	11 406	0.000	0.030
	5	Recent bottleneck	475 132	39 395	1 143	9 089	11 703	0.011	0.500
	6	Two bottleneck events	499 937	233395	22 843 897 5 181	5 308 65 838	116 809 11 517	0.007	0.170
Necrosyrtes monachus monachus	1	Stable	184 849	93 941	8 423	49 248	115 320	0.072	1.000
	2	Ancient expansion	26 667	148 688	9 569	75 806	115 955	0.002	0.240
	3	Ancient bottleneck	334 4875	81 819	8 727	45 535	115 285	0.047	0.990
	4	Recent expansion	77 778	197 195	881	4 299	11 527	0.037	0.980
	5	Recent bottleneck	344 153	81 820	892	7 702	11 528	0.046	1.000
	6	Two bottleneck events	381 246	221 376	11 209 893 5 055	75 837 77 097	115 999 11 503	0.003	0.990

Table Key: * NANC – Number of Ancestral individuals *NCUR – Number of current individuals,[#]yrs – Actual time of estimated occurrence, calculated from generational input time, Lower and Upper bound

Table 2.6: Approximate Bayesian computation (ABC) posterior estimations test the gene flow between the two subspecies of Hooded vulture (*Necrosyrtes monachus*) for different models and time frames. These models included two unidirectional gene flow both ways and bi-directional gene flow.

Population	Scenario	Description	*NANC	*NCUR		Time (yrs[#]) LB		Time (yrs[#]) Mode		Time (yrs[#]) UB		Marginal density	p-value
Both subspecies are experiencing a bottleneck event	7	Unidirectional migration (S-N)	30 308	90 855	33 250	12 731		51 143		71 873		3.307×10^{-27}	0.030
	8	Unidirectional migration (N-S)	30 305	99 870	36 315	12 620		32 228		71 699		1.825×10^{-27}	0.030
	9	Bidirectional migration	30 305	93 866	33 307	12 812	12 562	51 143	40 635	71 790	71 604	1.569×10^{-26}	0.220
	10	Unidirectional migration (S-N)	35 357	93 793	33 295	14 836		18 841		25 083		1.447×10^{-27}	0.030
	11	Unidirectional migration (N-S)	30 303	87 696	36 299	14 047		19 812		25 106		3.800×10^{-27}	0.180
	12	Bidirectional migration	35 358	90 752	33 278	14 833	14 838	21 268	21 146	25 086	25 065	6.678×10^{-28}	0.060
	13	Unidirectional migration (S-N)	35 355	84 840	33 207	31 811		72 054		100 054		1.547×10^{-25}	0.120
	14	Unidirectional migration (N-S)	35 356	102 769	36 295	31 568		63 167	63 975	99 872		6.982×10^{-28}	0.020
	15	Bidirectional migration	30 305	93 747	36 353	31 705	31 377	79 326	52 663	100 044	99 659	7.511×10^{-28}	0.040
	16	Unidirectional migration (S-N)	30 310	84 694	33 300	20 497		71 605		99 181		3.265×10^{-27}	0.120
	17	Unidirectional migration (N-S)	30 304	102 697	39 382	20 645		51 160		98 891		7.700×10^{-27}	0.130
	18	Bidirectional migration	35 357	105 850	36 315	20 541	20 598	62 312	52 089	99 019	99 067	2.440×10^{-26}	0.020

Table Key: * NANC – Number of Ancestral individuals *NCUR – Number of current individuals, [#]yrs – Actual time of estimated occurrence, calculated from generational input time, Lower and Upper bound

Discussion

In this study, the validity of the two Hooded vulture subspecies was investigated by analysing data from thirteen microsatellite markers for 42 individuals sampled across the continental distribution of the species. These microsatellite loci have been used in recent studies of other African vultures which allows for good comparison. The levels of heterozygosity observed in Hooded vultures (*N. monachus* $H_o = 0.451$, *N. m. monachus* $H_o = 0.485$ and *N. m. pileatus* $H_o = 0.424$) were similar to that recorded for Cape vultures (*Gyps coprotheres,* $H_o = 0.380$) (Kleinhans and Willows-Munro, 2019) and Bearded vultures (*Gypeatus barbatus* $H_o = 0.400 – 0.480$) (Streicher et al., 2021). These observed heterozygosity values are lower than those recorded for the Eurasian Griffon vulture (*Gyps fluvus*) ($H_o = 0.530 – 0.590$) and this highlights the genetic loss faced by African vulture populations due to population declines caused by anthropogenic threats (Davidović et al. 2020).

Phylogeographic structure of Hooded vulture

Population structure analyses did not support the current subspecies classification. Instead, our genetic data support a phylogeographic pattern of isolation-by-distance ($r = 0.244$). This finding is supported by previous studies done by Barlow et al. (2021) and Mundy (2021). Barlow et al. (2021) looked at the cranial biometrics of Hooded vultures and found that there was no significant difference in cranial measurements between the two subspecies, and that the only difference was in bill width. Mundy (2021) measured wing, tail and bill lengths and saw an increase in size due to cline in the species. Thus, both concluded the differences seen between the two subspecies are in fact size cline and not speciation and that changes seen between the two proposed subspecies are adaptations to different habitats and altitudes. The change in size is speculated to be a result of various factors, Mundy (2021) speculates that the increase in wing size could be a result of soaring vultures needing bigger wings to soar at higher altitudes. Mundy (2021) clearly states that these changes are not a result of the species being subjected to Bergman's rule.

Individuals from each locality sampled formed a genetic lineage, and the same patterns of high population differentiation have been seen in wide ranging species such as Bearded vultures and White-tailed eagles (*Haliaeetus albicilla*; Godoy et al., 2004; Hailer et al., 2007; Streicher et al., 2021). In contrast, Cape vultures showed very shallow genetic structuring (Kleinhans and Willows-Munro, 2019). The high population differentiation seen in Hooded vultures could be a result of reduced dispersal capability of this species due to a smaller home range compared

to larger vulture species that tend to show shallower population differentiation due to higher dispersal rates (Kleinhans and Willows-Munro, 2019; Suárez et al., 2022).

Demographic history of Hooded vultures

BayesAss indicated contemporary migration between the two subspecies, however, this migration is unidirectional. ABC analysis, however, indicated ancestral bidirectional migration between the two subspecies. The bidirectional migration indicated by ABC modelling occurred around 51 142 and 40 625 years ago. This migration occurred around the same period as when the climate was similar to that of the Last Glacial Maximum, which also showed sea levels 75m below present-day sea levels.

Taking into consideration the EDENetwork, we speculate that *N. m. monachus,* a more coastal species and urban exploiter (Barlow et al., 2022; Barlow and Fulford, 2013), migrated down the coastline into Angola moving within the distribution range of *N. m. pileatus*. This migration rate is high for a mobile species with a smaller home range compared to larger vulture species. Hooded vulture migration rates are low compared to the migration rates of Bearded vultures (*Gypaetus barbatus*), which range between 15%-28% for some populations (Streicher et al., 2021). These levels of migration between the two subspecies indicate that gene flow between subspecies is not restricted.

The ABC scenarios indicated that the two subspecies have experienced different demographic events. *Necrosyrtes monachus monachus* show clear signs of recent bottlenecks. This coincides with the majority of the trends seen in the vulture population due to anthropogenic factors and influence by the Bantu expansion similar to other savanna species such as rhinos (*Ceratotherium simum;* Çakmak et al., 2019; Davidović et al., 2020; Kleinhans and Willows-Munro, 2019; Moodley et al., 2018; Streicher et al., 2021).

The historic bottleneck event seen in *N. m. pileatus* that took place around 77 067 years ago occurred due to a colder climate and similar vegetation seen during the Last Glacial Maximum. This shift in vegetation consisted of more extensive forest in central Africa and moister conditions in the Eastern highlands of Africa, which caused a decrease in savanna habitats (Adams and Faure, 1997; Ehrmann et al., 2017; Hoag and Svenning, 2017; Miller and Gosling, 2014). Thus, indicating that when the savanna decreases the population size decreases due to a lack of suitable habitat.

Despite the lack of support for the two subspecies, it is seen that the two groups have experienced different demographical changes that could be linked to changed linked to changes in the climate. Conservation programs should incorporate these local differences into management plans to better conserve the species as a whole. These management plans should incorporate captive breeding programs for each country with rehabilitated birds that cannot be released back into the wild. Yearly population counts should be done for each country as census data for this species is lacking, and habitat restoration should be considered for some countries.

Chapter 3

High conservation importance of range-edge populations of Hooded Vultures (*Necrosyrtes monachus)*

Rynhardt Le Roux, Justus P. Deikumah, Lindy J. Thompson, Bettine van Vuuren, Sandi Willows-Munro

Abstract

Hooded vultures (*Necrosyrtes monachus* Temminck, 1823), like many vulture species globally, are experiencing rapid population declines due to anthropogenic factors such as poisonings, human persecution, and habitat loss and degradation. The Hooded vulture is widespread across sub-Saharan Africa. Although it is considered one of the most abundant vultures in West Africa, in East and South Africa, this vulture species is less common, with the population at the most southern edge of the distribution (South Africa and Eswatini) estimated at only 100-200 individuals. Worryingly, the distribution of Hooded vultures has contracted dramatically in southern Africa, with breeding populations largely confined to protected areas such as the Kruger National Park. This study aimed to investigate the genetic diversity of the southern African range-edge population and assess if the recent contraction in the distribution has resulted in the population experiencing a genetic bottleneck. Sixteen microsatellite loci were used for amplification in individuals (n = 30) collected along the Olifants River in the Greater Kruger National Park area. The genetic diversity present in the South African population was compared to samples (n = 30) collected in Ghana, where Hooded vultures are more abundant. Contrary to expectations, the South African peripheral Hooded vulture population showed higher levels of heterozygosity (H_O = 0.495) than the Ghanaian population (H_O = 0.315). Both populations showed no signs of recent bottleneck events when testing using a stepwise mutation model and a two-phase mutation model. The South African population showed a similar level of heterozygotic excess (H_X= 0.998) for the stepwise mutation model compared to the Ghanaian population (H_X= 0.999). The two-phase mutation model shows a difference, as the Ghanaian population shows higher levels of heterozygotic excess (H_X= 0.997) compared to the South African population (H_X= 0.892). These populations both show high levels of inbreeding and relatedness between individuals. The South African population shows similar levels of inbreeding (LynchRd mean = 0.187; TrioML mean = 0.237) compared to the Ghanaian population (LynchRd mean = 0.164; TrioML mean = 0.303). Levels of relatedness between individuals were also similar between the South African and Ghanaian populations

(Ghana: LynchRd mean = 0.038; TrioML mean = 0.100. South Africa: LynchRd mean = 0.036; TrioML mean = 0.112). The data suggests that despite being a small peripheral population, the South African Hooded vultures show a similar level of genetic diversity in comparison to a population sampled from the core of the species distribution. This supports conservation efforts in the Southern African region.

Key words: Hooded vulture, Range-edge population, South Africa, Ghana, *Necrosyrtes monachus*

Introduction

The numbers of Hooded vultures (*Necrosyrtes monachus* Temminck, 1823), like many vulture species in Africa, have in recent years declined drastically (Ogada et al., 2016; Ottinger et al., 2021; Rushworth et al., 2018; Thompson et al., 2017a; Watson et al., 2004), and the species is listed as Critically Endangered by the International Union for Conservation of Nature (BirdLife International, 2022). Vulture declines have been linked to anthropogenic factors such as poisoning (intentional and indirect), the bushmeat and traditional medicine trade, and habitat destruction or degradation (Buechley and Şekercioğlu, 2016; Buij et al., 2016; Henriques et al., 2020; McKean et al., 2018; Ogada et al., 2016, 2012; Petrozzi, 2018; Saidu and Buij, 2018; Thompson et al., 2017b).

The Hooded vulture is widespread in sub-Saharan Africa and is considered one of the most abundant vultures in West Africa, with over 43 000 individuals recorded in Guinea-Bissau alone (Henriques et al., 2018, 2017). Flocks of about 500 birds have been seen around the slaughterhouses in Ghana (Gbogbo et al., 2016). In East and South Africa, this vulture species is less common, with the South African and Eswatini populations estimated to consist of only 100-200 mature individuals (Taylor et al., 2015). With Southern Africa being the edge of their distribution, the Hooded vulture is limited to areas in Botswana, South Africa and Zimbabwe. In Botswana and Zimbabwe, most Hooded vultures are concentrated around the Okavango Delta and the Zambezi River (BirdLife International, 2017; Monadjem et al., 2016; Mundy et al., 1992; Ogada and Buij, 2011; Roche, 2006; Thompson et al., 2020, 2017a, 2017b) In South Africa, these birds are predominantly restricted to conservation areas in and around the Greater Kruger National Park (Monadjem et al., 2016; Ogada and Buij, 2011; Roche, 2006; Thompson et al., 2020, 2017a, 2017b).

There is evidence that African Hooded vulture populations have declined rapidly over the last decade (Ogada et al. 2016). Studies using data from West Africa collected between 1969/70 and 2003/4 have suggested that populations have undergone an estimated annual decline of -2.8%, equivalent to a reduction of 68% over three generations (Thiollay, 2006). Although declines of Hooded vultures in southern African countries seem less drastic (Figure 3.1), the current low density of Hooded vultures along the southern edge of the distribution may be linked to declines in earlier decades, and this peripheral population may represent a remnant of a once larger distribution (Tarboton and Allan, 1984).

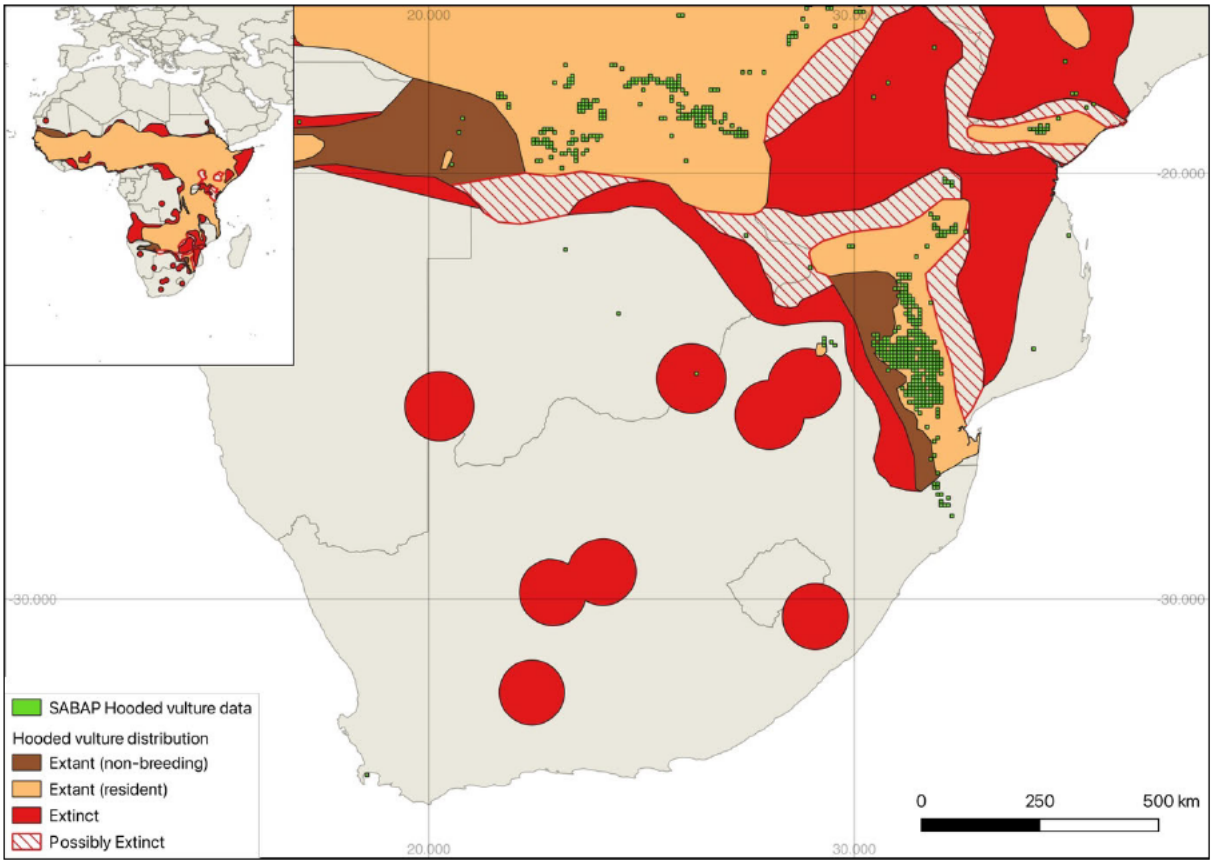

Figure 3.1: The current and historical distribution of Hooded vulture (*Necrosyrtes monachus*) in southern Africa. Inset is the distribution of Hooded vulture across Africa, with supplementary distribution of Hooded vultures (indicated in green) within South Africa (As provided by the South African Bird Atlas Project, BirdLife International, 2022).

Populations at the edge of a species distribution are generally smaller and often geographically isolated. Small, isolated populations are often characterised by low genetic diversity and elevated levels of inbreeding (Nowell et al., 2022), which may lead to reduced population fitness. Empirical studies of the genetic diversity present in peripheral populations of both plants (Eckert et al., 2008; Sagarin and Gaines, 2002) and animals (Cameron and Hargreaves,

2020; Wang et al., 2002; Winker et al., 2000) have not always supported this hypothesis with some studies finding that range-edge populations remain genetically similar to core populations (Eckert et al., 2008; Sagarin and Gaines, 2002). Lack of range-edge genetic differentiation may, in part, be due to species-specific characteristics (Gibson et al., 2009), such that highly mobile species may be able to reduce the effects of genetic drift in peripheral populations by maintaining connectivity.

If this holds true, then range-edge populations of highly mobile species, such as the Hooded vulture, should not be genetically different from populations found in the core of the distribution. Although the value of conserving these edge populations, such as the South African Hooded vulture, may be disputed due to lower genetic diversity (Gibson et al., 2009; Nowell et al., 2022), studying peripheral populations is important for understanding range shifts and adaptive capacity under climate change. These edge populations could potentially also retain unique genetic diversity, which helps with the adaptation of species to different environments (Bunnell et al., 2004; Eckert et al., 2008; Leppig and White, 2006; Nowell et al., 2022).

In this study, this hypothesis is tested by comparing the genetic diversity of two populations of Hooded vultures – one from the core range of the species (Ghana) and a peripheral population from South Africa. Both populations will also be assessed for bottleneck events to provide evidence for a potential range and distribution decrease. Inbreeding and relatedness will also be tested to determine if individuals belonging to the peripheral South African population are being exposed to the detrimental effects of inbreeding depression.

Materials and methods

Sampling

Moulted feathers under nests were collected from two populations: One from the core range of the Hooded vulture (Ghana) with high population densities and one from the peripheral South African population with low population density. In Ghana, 30 samples were collected from the central and eastern regions of Ghana. Sampling occurred in 2017 at the University of Cape Coast farms in Ghana and the surrounding University Lecturers village in the Central Region of Ghana, and then in the Zongo Hohoe suburb and St. Francis College campus in the Eastern region of Ghana (Figure 3.2). The transect between the two sampling regions was 294.66 km. The South African samples were collected along a 94.06 km transect following the Olifants

River, and then two individuals were sampled 67.84 km and 163.67 km away from the original sampling site in the Limpopo Province between 2015-2019 (Figure 3.2). The DNA yield of feathers varies significantly in relation to the calamus size and condition (Horváth et al., 2005; Segelbacher, 2002); for this reason, larger feathers with intact calamus and umbilicus were preferred for DNA extraction. These feather samples collected under each nest were placed in individual sterile, airtight envelopes with silica gel and stored at room temperature until DNA extraction. Ethical clearance was granted by the Animal Research Ethics Committee from the University of Kwazulu-Natal (AREC/022/020). Due to this species being listed as Critically Endangered, a Section 20 permit (ref 12/11/1/5(2104NC)) and veterinary permit (ref MJV GR 76/22) were also obtained for the collection and movement of samples.

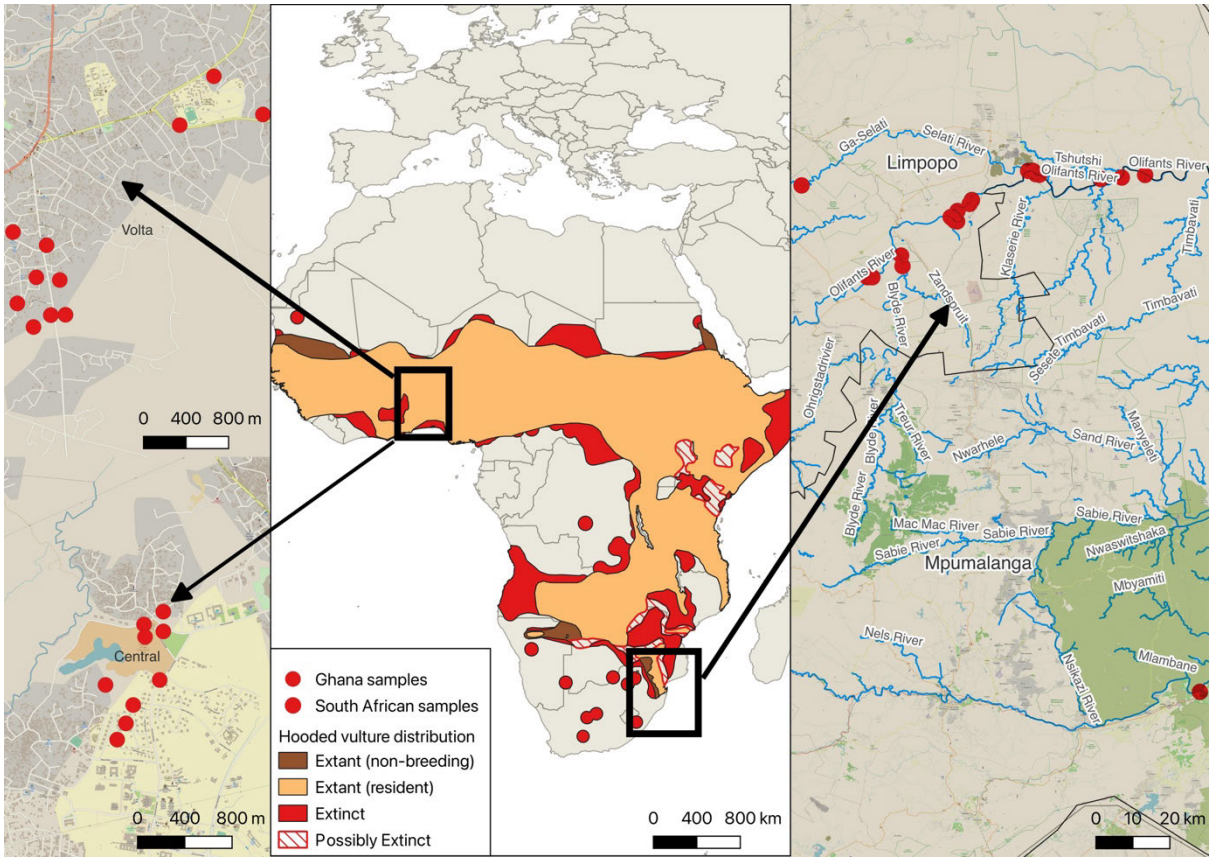

Figure 3.2: The sampling localities for Hooded vulture (*Necrosyrtes monachus*) in Ghana and South Africa. The eastern region top left corner and the central region in the bottom left corner and in southern Africa on the right. Then the full pan-African distribution of Hooded vulture indicated in the middle.

DNA extraction and microsatellite amplification

The E.Z.N.A® Tissue DNA kit (Omega Bio-Tek, Germany) was used for all DNA extractions. Only the tip of the calamus containing a blood clot was used for DNA extraction (Horváth et al., 2005; Segelbacher, 2002). Small modifications to the standard E.Z.N.A® kit protocol were made to ensure sufficient DNA yield from feathers. The modifications included the incubation of samples in a buffer with proteinase K for 24 hours in a water bath (56°C). Every hour the samples were vortexed. The lysate was then incubated in B3 buffer for 45 minutes at 70°C; the final volume of 100 µl pre-warmed Buffer BE was added to the spin column and centrifuged then reheated after the first centrifuge for 5 minutes, there after reapplied to the membrane for a final centrifuge step.

Sixteen microsatellite loci were amplified in this study (Table 3.1). These markers were chosen from a suite of markers used in previous studies of *Gyps coprotheres* and other vulture species, including Hooded vulture (Kleinhans and Willows-Munro, 2019). The loci were amplified in five multiplex reactions using the Multiplex TEMPase PCR Kit (AMPLIQON, Denmark), and each forward primer was fluorescently labelled. Each 10µl reaction consisted of 1-3µl of DNA template, 5µl of Multiplex TEMPase mix and 0.2µl of 0.2 µM for each primer and 3.6-1.6µl purified water to obtain the final volume of 10 µl. Thermocycler parameters were as follows: initial denature at 94°C for 15 min, then followed by 94°C for denaturing, 60°C for annealing and then 72°C for elongation for a total of 35 times. Then a final elongation at 72°C for 10 min and an infinite hold at 4°C until samples were removed from the thermocycler. Negative controls were included to ensure that no contamination of reagents occurred. Amplified products were sent to the Central Analytic Facility (CAF) at Stellenbosch University, South Africa, for fragment analyses using a ABI3730xl machine. The genotype scoring was done using GeneMarker v2.6.3 (Soft Genetics). To ensure genotype consistency, all samples were re-amplified multiple times (up to seven times) and cross-referenced to ensure consistency.

Table 3.1: List of loci used in genetic analysis of Hooded vulture (*Necrosyrtes monachus*), primers used for amplification, fluorescent dyes used for tagging the forward primers, annealing temperature (T_m) used in each reaction for the amplification of specific microsatellite loci with the combination of loci amplified together in multiplex reactions.

Locus	Primers	Dye	T_m	Motif	Multiplex
BV2	F: CAGCATGTTATTTTGGCTGC R: TTGCTAAACCGGTTAGAAGTTG	HEX	60	CA_{11}	A

BV6	F: AATCTGCATCCCAGTTCTGC	HEX	60	CA_{11}	D
	R: CCGGAGACTCTCAGAACTTAAC				
BV8	F: TGGCATGCTGCTATGAGAAC	FAM	60	CA_{11}	C
	R: GTGCTTTGCATGCTTTTACTC				
BV9	F: ATCTAGGGACATCGAGGAGC	HEX	60	TA_6 CA_{11}	D
	R: ACAGGGATGCAGGTAAGCC				
BV11	F: TGTTTGCAAGCTGGAGACC	HEX	60	CA_{22}	B
	R: AAAAGCCTTGGGGTAAGCAC				
BV12	F: TCAGGTTTTGACGACCTTCC	FAM	60	CA_{15}	C
	R: GTGGTAACGGAGGAACAAGC				
BV13	F:AAAACAGAGTTTTCACATTTTCATAAG	FAM	60	CA_{16}	A
	R: TTCAGGAAACAGAAGCATGAAC				
BV17	F: TGATGTGCAGATGCGTGAC	HEX	60	CA_{11}	C
	R: GGACTCTGATGAAGCCAAGC				
BV20	F: GAACAGCACTGAACGTGAGC	HEX	58	CA_{13}	E
	R: GTTTCTCCTGACAGTGAAATAACTC				
Gf3f3	F: GATCTTTCCCCTTCTGTG	TET	60	CT_{10}	E
	R: TTCGTGCAGTGATGCTGGTG				
Gf3H3	F: GTAGAATAATTTGCTCCTGG	FAM	60	CT_{12}	D
	R: GTGAAGGCACCTCATAGACA				
Gf8G	F: TGAGCAGGTGAGTCCAGAAG	FAM	60	CT_8C TC_2	B
	R: GCTCTCCTGTCATCTTGCAT				
Gf9C	F: GGTGGACATTACATACACTG	HEX	60	TC_{10}+CT_9 C CA_5T AC_4	A
	R: CAAGGAATCTGGACTACTAA				
Gf11A4	F: GATCCCTTCCAACCGAAAAT	HEX	60	$CTCTT_{17}$	C

	R: TGGTGACCAACGGAAGTGTG				
BV14	F: GGCAGTGTGGAGCCTACATC	Fam	60	CA_{16}	C
	R: CTCCAGGGTCCTTGTTTGC				
BV5	F: GTTCTGAGGGTAGAGGGACTG	Tet	58	CA_{17}	E
	R: GCTGAGCAGCTTCAGAAAGTC				

Analyses of genetic variation

Cervus v3.0.7(Kalinowski et al. 2007) was used to check that the same individual was not sampled twice and to determine the polymorphic information content (PIC) for each locus. Null allele frequencies were estimated using FreeNA (Chapuis and Estoup, 2007).

Genepop v4.2 (Raymond and Rousset, 1995) was used to test for deviation from Hardy-Weinberg equilibrium. Genetic diversity was measured in each of the two populations by estimating the number of alleles, observed heterozygosity, and unbiased expected heterozygosity in GenALEx v6.502 (Peakall and Smouse, 2006). FSTAT v2.9.3.2 (Goudet, 1995) was used to determine the allelic richness for both populations. The number of private alleles was determined in HP-RARE v 1.0 (Kalinowski, 2005).

Population structure

Bayesian assignment tests were performed in STRUCTURE v2.3.4 (Hubisz et al., 2009) on each population separately to determine if population structure was present. Ten independent runs were performed consisting of 500 000 Markov chain Monte Carlo (MCMC) replicates with a burn-in of 50 000 and a proposed number of genetic clusters (K) from 1 to 10. The admixture ancestry model with correlated allele frequencies was selected for each run. The optimal number of genetic clusters was estimated using the Evanno method (Evanno et al., 2005) in STRUCTURE Selector (Li and Liu, 2017). Individual membership probabilities (Q-values) were estimated in STRUCTURE Harvester (Earl & von Holdt 2012), and bar plots were created in Pop-helper (Francis, 2017).

Demographic history

Approximate Bayesian Computation estimation (ABC) modelling was used to test a number of different demographical scenarios (Beaumont et al., 2002) using ABCToolbox v. 1.1

(Wegmann and Excoffier, 2010). This modelling was conducted on each population separately. In ABC, a coalescent model is used to generate a reference suite of simulations that are compared to real data based on summary statistics. Simulated datasets that produce summary statistics more similar to those of the real data are considered to be generated by models with higher likelihood. This comparison between simulated and real data is made using a random forest algorithm. ABC estimates the time of events according to generational time. Therefore recent demographical changes for this study incorporated a time frame from 1 year to 12 500 years ago, and the ancient demographical changes used a time frame between 1 and 125 000 years ago. This specific time for recent demographical changes was selected based on changes in the climate that led to changes in forest cover across Africa and falls within a period just after the last glacial maximum (Adams and Faure, 1997; Elenga et al., 1994). Given that Hooded vultures occur in savannah and grasslands, the expansion and contraction of these biomes are expected to impact the demographic histories of this species. Then this time frame was also selected to incorporate demographical changes that occurred within the last 1 000 generations. The wide priors were selected for the ancient demographical changes to ensure that all climatic and non-climatic forces that could influence the populations was accounted for.

In this study, simulations were conducted under six different scenarios. A stable population was modelled with no expansion or contraction of the population (Scenario 1). This is used as the null model. Two population expansion models were tested: One simulating a recent population expansion (Scenario 4) from one to 961 generations ago and one modelling an ancient expansion (Scenario 2) from one to 9 651 generations ago. Two bottleneck events were also modelled: One simulating a recent bottleneck (Scenario 5) from one to 961 generations ago and one modelling an ancient bottleneck (Scenario 3) from one to 9 651 generations ago. Finally, a double bottleneck event was modelled, testing a recent and ancient event simultaneously with similar generational times as the recent and ancient models tested previously (Scenario 3 and 5). Therefore, to calculate the actual time of these events, generational time was converted into time in years, 13 years (BirdLife International, 2022). These models were run for 200 000 simulations, and a total of 5 000 simulations were used for the parameter and posterior probability estimation.

The results of the ABC analyses were compared to those estimates produced using the programs BOTTLENECK v1.2.02 (Piry et al., 1999) and M-P-Val and Critical_M. BOTTLENECK tests for evidence of heterozygosity excess (H_x) to determine if and when

bottleneck events occurred in each of the populations. Analyses were done using two mutation models: the more conservative stepwise mutation model (SSM) and the two-phase model (TPM). The conservative SSM and TPM approach incorporates a 90% stepwise mutation (Dussex et al., 2015; Garza and Williamson, 2001) with a variance of 12 (Piry et al., 1999) to ensure that the full range of multi-step mutations was accounted for in the natural population (Di Rienzo et al., 1994). Two different statistical tests were performed to detect bottlenecks. The Wilcoxon sign-rank test was performed to detect recent changes in effective population size, by testing for a changed in heterozygous excess (H_X) and heterozygous deficiency (H_d).. In particular, this test is useful in detecting higher heterozygosity levels (in comparison to a population at mutation-drift equilibrium) in populations that have undergone a fairly recent bottleneck (a population decline in a few generations). The mode shift test detects a genetic change caused by a population decline in a few generations. The M-Ratio test was performed as it detects bottlenecks that happened up to 100 generations back (Garza and Williamson, 2001; Zachariah Peery et al., 2012). This analysis was performed using M-P-Val and Critical_M (Garza and Williamson, 2001). Pre-Bottleneck Ne values were set at 100, 5000 and 50000 individuals, with a mutation rate of 0.0005. Two TPM mutation models were selected. The first parameters are being used more widely with a single-step mutation of p_s = 0.88 and Δg = 2.8 as a multi-step mutation rate. Then another parameter set that is more conservative with p_s = 0.9 and Δg = 3.5 was tested (Garza and Williamson, 2001). The effective population size for each population was determined using NeEstimator v2.01 (Do et al., 2014).

To estimate the relatedness of individuals in each population and to calculate inbreeding, the program Coancestry v1.0.1.9 (Wang, 2011) was used. This program offers seven different models to estimate relatedness and four models for inbreeding (calculating inbreeding via internal relatedness). Wang's unbiased estimator was used as it is appropriate for a population where inbreeding is suspected (Wang, 2011). The internal individual inbreeding coefficient was calculated using LynchRD. These runs were conducted for each population without first-order kin accounting for inbreeding and an error rate of 0.0005.

Results

All 60 samples were successfully genotyped, and no samples were found to come from the same individual or close relative. Unfortunately, three markers (BV12, GF11A4, Gf3f3) did not amplify well in individuals from the Ghanaian population, and so for this population, only 13 microsatellites were used in analyses. In the South African population, two markers (BV14,

BV5) did not amplify in all individuals, and so only 14 microsatellite markers were used for analyses of this population.

Genetic diversity

The mean null allele frequency for the South African is 10.1% (0.101) and Ghanaian 9.3% (0.093) (Table 3.2). In the South African population, five loci were identified (BV2, BV13, BV17, BV9, BV20) with elevated levels of null alleles (Appendix 3.1). While in the Ghanaian data set, six loci were identified (BV2, Gf8G, BV8, Gf11A4, BV20, Gf3f3) with higher levels of null alleles. The number of alleles ranged from 4.000 – 14.000 for the South African population, while the number of alleles in the Ghanaian population ranged from 1.000 to 12.000.

Both populations indicate signs of heterozygosity deficiency, with Ho for the South African population being 0.495 and that for Ghana being 0.315. When estimating allelic richness among the populations, South Africa showed an allelic richness of 7.285, and Ghana showed 5.068.

Table 3.2: Summary showing the statistics of (n = 60) Hooded vulture (*Necrosyrtes monachus*) from South Africa (n = 30) and Ghana (n=30) genotyped in the current study. The number of alleles (A), number of effective alleles (N_e), null allele frequency (N_o), private alleles (A_P) , allelic richness (A_R), observed heterozygosity (H_o), expected heterozygosity (H_e), unbiased expected heterozygosity (uHe), deviation from Hardy-Weinberg equilibrium (HWE) p-value, polymorphic information content (PIC), and inbreeding coefficient (F) of each population is given.

Location	A	Ne	N_0	A_P	A_R	H_o	H_e	uHe	HWE	PIC	F
South Africa	7.357	3.664	0.101	4.630	7.285	0.495	0.621	0.632	0.072	0.692	0.254
Ghana	5.692	2.862	0.093	3.500	5.068	0.315	0.418	0.428	0.162	0.398	0.335

Demographic history

The optimal number of genetic clusters for both the South African and Ghanaian Hooded vulture populations was one, and there was no evidence of strong population structure in either population.

ABC analyses (Table 3.3 and Appendix 3.2-3.4) found that for the South African population, the most likely scenario was a recent as well as ancient expansion (p-value = 0.970) compared to the Ghanaian population that only experienced a recent population expansion (p-value = 1.000). The difference between these events for both these populations is the estimated time when these expansions occurred. For the Ghana population, the estimated time for this recent expansion was around 4 044 years ago, and for the South African population, the recent expansion was around 5 181 years ago Both the Ghanaian and South African population show similar effective populations sizes after these expansion events.. The Ghanaian population shows an effective population size of 194 229 individuals right after its expansion event. The South African population showed an effective population size of 184 935 individuals after its expansion event. The ancestral expansion event seen for the South African population occurred around 77 066 years ago and showed an effective population size of 197 055.

Table 3.3: Approximate Bayesian computation (ABC) posterior estimations for three demographic scenarios for the South African and Ghanaian Hooded vulture (*Necrosyrtes monachus*) populations. Six demographic scenarios were tested including a stable population, an ancestral bottleneck, an ancestral expansion, a recent expansion, a recent bottleneck and then, lastly, two bottlenecks at different time periods.

Population	Scenario	Description	NANC*	NCUR*	Time (yrs[#]) lower bound	Time (yrs[#]) Mode	Time (yrs[#]) upper bound	Marginal density	p-value
South Africa	1	Stable	70 709	200 001	10 113	60 610.290	114 895	0.011	0.850
	2	Ancestral expansion	23 031	197 055	16 526	77 067	119 950	0.039	0.970
	3	Bottleneck	143 876	69 700	15 265	89 680	119 950	0.002	0.600
	4	Recent expansion	42 425	184 935	1 148	5 181	11 737	0.047	0.970
	5	Recent bottleneck	132 082	66 668	769	3 669	11 232	0.002	0.550
	6	Two bottleneck events	204 502	115 658	11 246 853	82 139 4 927	11 5918 11 468	0.002	0.280
Ghana	1	Stable	45 455	84 849	9 499	63 128	115 528	0.029	0.980
	2	Ancestral expansion	22 727	160 699	9 042	51 832	115 507	0.062	0.980
	3	Bottleneck	82 441	39 395	9 176	74 581	115 529	0.002	0.750
	4	Recent expansion	33 334	194 229	871	4 044	11 527	0.066	1.000
	5	Recent bottleneck	92 082	39 396	889	7 827	11 530	0.002	0.900
	6	Two bottleneck events	154 830	97 123	10 806 869	72 033 5 304	115 793 11 526	0.000	0.260

Table Key: * NANC – Number of Ancestral individuals *NCUR – Number of current individuals, [#]yrs – Actual time of estimated occurrence, calculated from generational input time.

A population bottleneck could negatively affect the genetic health of a population. In Table 3.4, the population bottleneck analysis for the South African and Ghanaian Hooded vultures is seen. The South African population shows a heterozygosity excess for both the stepwise mutation model (SMM) and the two-phase mutation model (TPM) Wilcoxon test similar results are seen for the Ghanian population with a heterozygosity excess being seen for both tests. The heterozygosity deficiency to heterozygosity excess ratios also shows deviation from the expected ration (expected ratio 1:1), for the South African population, with 11:1 and 9:3 for both tests. The Ghanian population also indicated this deviation from the expected ration; however, the p-value = 0.158 and there for cannot be seen as statistically significant. Thus, it can be concluded that both populations have not undergone a recent population bottleneck.

The M-Ratio indicated a bottleneck event that happened up to 100 generations ago for both populations as a M ratio below 0.7 indactes a bottleneck event and a M ratio above 0.8 indcates a stable population. As for both parameter sets and the three selected Ne sizes, the M < Mc. With M for the South African population being M (M ratio = 0.367) and Mc for the actual Ne for South Africa being (M ratio = 0.527) and (M ratio= 0.452) for the more conservative parameter set. Similar results are seen for the Ghanaian population where the M (M ratio = 0.398) and the Mc values being significantly higher than that (M ratio = 0.420) only deviating from this on the parameter for the more conventional parameter set. The

The effective population size for both populations with a confidence interval of 95% was Ne = 15.2 individuals. Thus, the ratio for effective population size to census population size (Nc = 200 ; BirdLife International, 2017; Roche, 2006; Frankham, 1995) for South Africa would be Ne/Nc = 0.076. The ratio for effective population size to census population size is Nc = 500 based on estimates of birds that have been surveyed. Thus, the ratio is Ne/Nc = 0.030 for the Ghana population (BirdLife International, 2017; Gbogbo et al., 2016).

Table 3.4: Bottleneck results of the South African and Ghanaian Hooded vulture (*Necrosyrtes monachus*) populations. Two mutation models were used, the stepwise mutation model (SMM) and the two-phase mutation model (TPM). Parameters for a sign test, Wilcoxon signed ranked test, which tests for a heterozygous excess (H_X) and a heterozygous deficiency (H_d) and a Mode-shift test for the detection of the bottleneck are also represented. A Mode-Shift analysis was also done, and an M-ratio test was performed using two parameter sets.

Population	Wilcoxon test				Sign test				M-Ratio						Mode-Shift
	One-tailed for H_x		One-tailed for H_d		SMM		TPM		p_s = 0.88			p_s = 0.9			
	SMM	TPM	SMM	TPM	H_d: H_x	p-value	H_d: H_x	p-value	Δg = 2.8			Δg = 3.5			
South Africa	0.998	0.892	0.002	0.121	11:3	0.005	8:6	0.158	Θ=0,2 Ne = 100	Mc	0.527	Θ=0,2 Ne = 100	Mc	0.452	No
									Θ=10 Ne = 5000	Mc	0.543	Θ=10 Ne = 5000	Mc	0.456	
									Θ=100 Ne = 50000	Mc	0.421	Θ=100 Ne = 50000	Mc	0.349	
									M-Ratio		0.367	M-Ratio		0.367	
Ghana	0.999	0.997	0.001	0.004	11:1	0.000	9:3	0.021	Θ=0,2 Ne = 100	Mc	0.524	Θ=0,2 Ne = 100	Mc	0.447	No
									Θ=10 Ne = 5000	Mc	0.541	Θ=10 Ne = 5000	Mc	0.452	
									Θ=100 Ne = 50000	Mc	0.420	Θ=100 Ne = 50000	Mc	0.347	
									M-Ratio		0.398	M-Ratio		0.398	

To further understand the genetic health of the populations, their overall relatedness and inbreeding factors were estimated using both the LynchRD and TrioML method as these estimations have been shown to estimate relatedness and inbreeding better than other methods. Both these estimations are used as they implement different methods of determining relatedness and inbreeding and therefor gives a better overall picture as to what is happeing in the population. In Figure 3.3 for the South African population, LynchRd (Fig 3.3 B) and TrioML (Fig 3.3 A) relatedness tests show high levels of relatedness. The LynchRd has a mean = - 0.036, and for TrioML, the mean = 0.112; this shows that most individuals are related to each other for both these tests. Then further Fig 3.3 shows LynchRd (Fig 3.3 D) and TrioML (Fig 3.3 C) for the inbreeding coefficient that this population is starting to inbreed. The mean of both tests was 0.187 and 0.237, respectively. These mean values indicate how this population is starting to inbreed. Comparing that to the Ghanaian population, Figure 3.4 for LynchRd (Fig 3.4 B) and TrioML (Fig 3.4 A) relatedness tests shows high levels of relatedness. The LynchRd has a mean of - 0.038, and for TrioML, the mean is 0.100; this shows that most individuals are related to each other. Both the LynchRd (Fig 3.4 D) and TrioML (Fig 3.4 C) for the inbreeding coefficient (Fig 3.4) also show that this population is starting to inbreed, with means of 0.164 and 0.303 respectively. Thus, both these populations show signs of inbreeding and little to no gene flow between surrounding populations.

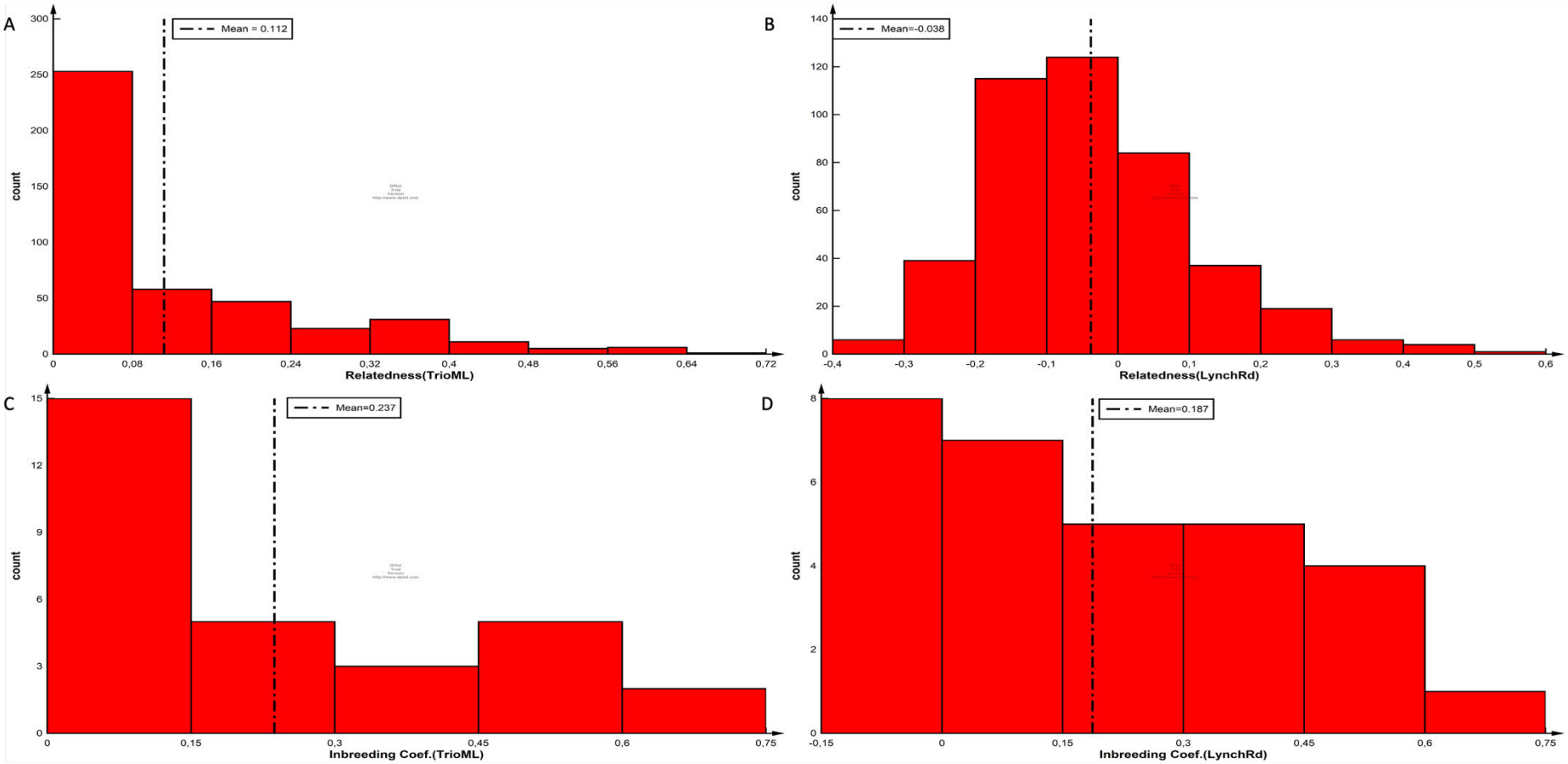

Figure 3.3: The TrioML inbreeding coefficient and relatedness (A-C) for the South African Hooded vulture (*Necrosyrtes monachus*) population is based on the number (count) at which the individuals share similar alleles. The same applies to the test LynchRd inbreeding coefficient and relatedness (B-D). The means for both the inbreeding coefficient and relatedness are represented by the dashed lines and these are 0.112, 0.237, -0.038 and 0.187

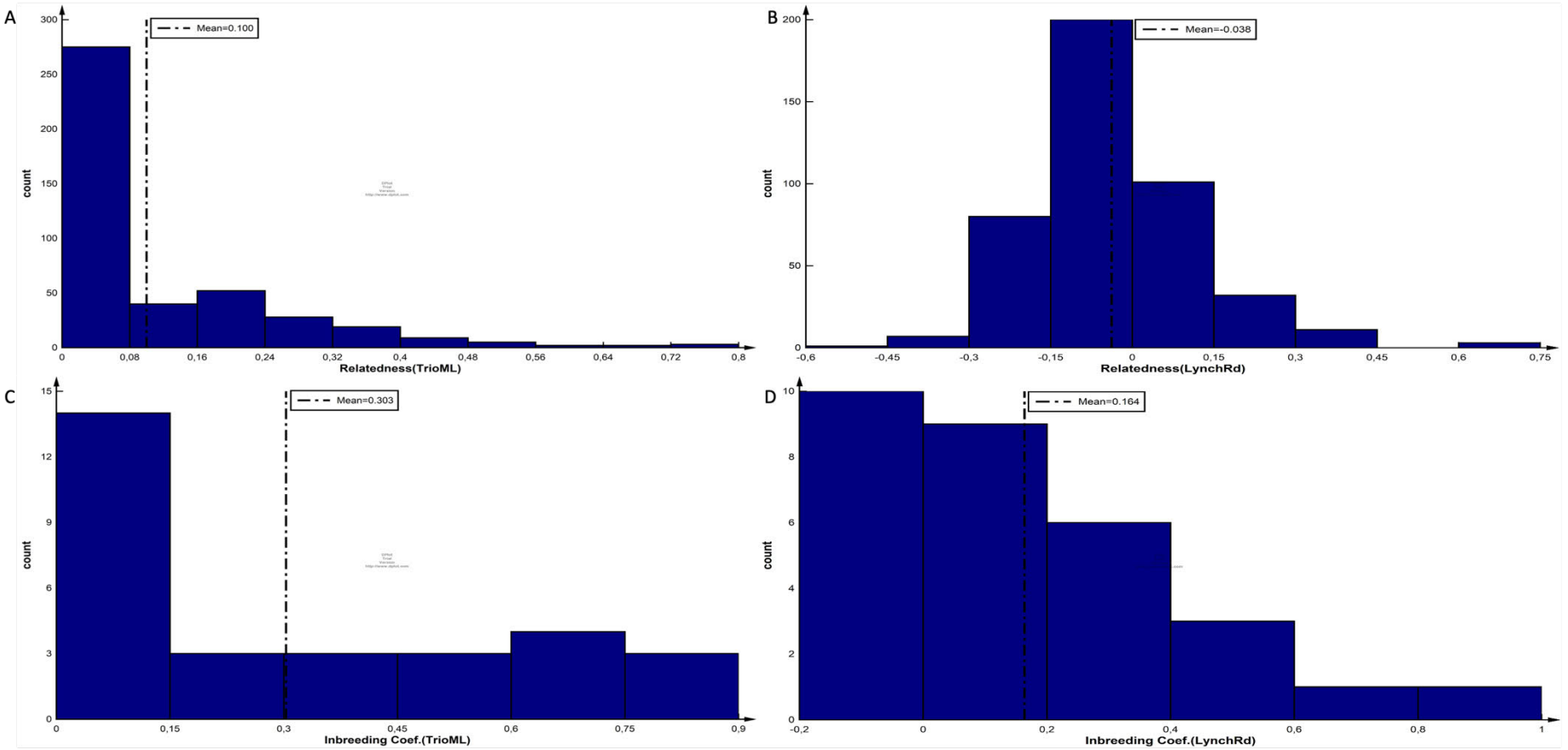

Figure 3.4: The TrioML inbreeding coefficient and relatedness test (A-C) for the Ghanaian Hooded vulture (*Necrosyrtes monachus*) population is based on the number (count) at which the individuals share similar alleles. The same applies to the LynchRd inbreeding coefficient and relatedness (B-D). The means for both the inbreeding coefficient and relatedness are represented by the dashed lines and these are 0.100, 0.303, - 0.038 and 0.164.

Discussion

The spatial distribution of the genetic variation present in populations of endangered species is a product of population collapse, bottlenecking events, fragmentation, and isolation of populations (Eckert et al., 2008; Furlan et al., 2012; Pironon et al., 2017). In particular, smaller peripheral populations at the extremes of a species' distribution are often not considered as important for conservation as they may be genetically depauperate. In contrast, other authors such as Bunnell et al. (2004), Leppig & White (2006), Eckert et al. (2008), and Nowell et al. (2022), have argued that the study of peripheral populations is important in understanding the range shifts and adaptive capacity under climate change (Bunnell et al., 2004; Eckert et al., 2008; Leppig and White, 2006; Nowell et al., 2022). In this study, the genetic diversity, and demographic histories of two Hooded vulture populations were compared. Hooded vultures are more common and occur at higher densities in West Africa (Henriques et al., 2018; Jallow et al., 2022, 2016). In contrast, the edge of range South African population is much smaller, with Hooded vultures in this region confined to protected areas in and around the Greater Kruger National Park (Daneel, 1984; Roche, 2006; Thompson et al., 2017b).

Using microsatellite data, it was found that the South African population is as genetically diverse as the West African population. Observed levels of heterozygosity for the South African population($H_o = 0.495$) and for the Ghanaian population ($H_o = 0.315$), are comparable to values found in Cape vultures (*Gyps coprotheres*, $H_o = 0.380$) (Kleinhans and Willows-Munro, 2019), Bearded vultures (*Gypaetus barbatus*, $H_o = 0.400\text{-}0.480$) (Streicher et al., 2021), and Griffon vultures (*Gyps fulvus,* $H_o = 0.530– 0.600$; Davidović et al. 2020). These studies all use a similar set of microsatellite markers. The Cape vulture, Bearded vulture and Hooded vulture populations show similar signs of low genetic diversity, compared to the Griffon vulture which indicated higher levels of genetic diversity.

The ABC analyses indicated that the South African populations had undergone two periods of expansion (77 067 and 5 181 years ago), while the Ghanaian population had just undergone a recent expansion (4 043 years ago). Around 5 000 years ago, the climate became more arid and drier similar to current climate conditions (Elenga et al. 1994, Ritchie 1994, Vincens et al. 1998); the central African forests reduced in size while savannah and grassland habitats became more widespread. This expansion also corresponds to the Bantu migration that started around the same time as the early domestication of animals for the use of agriculture (Bayon et al., 2012; Grollemund et al., 2015; Moodley et al., 2018; Patin et al., 2014). These people also had

access to iron age smelting technologies, and thus would have been able to hunt larger game (Bayon et al., 2012; Moodley et al., 2018). This hunting of larger game would have led to more carrion waste (and thus more available food for Hooded vultures which are near-obligate scavengers; (Barlow et al., 2022)), which could have indirectly led to this expansion in the vulture population as seen in the ABC models (Parra and Tellería, 2004).

Worryingly both the South African and Ghanaian populations show elevated levels of inbreeding and relatedness. Of particular concern is the elevated inbreeding recorded from Ghana. If this population is considered representative of the West African Hooded vultures, then the inbreeding is of conservation concern.

In conclusion, the genetic diversity of the small peripheral population in South Africa is not significantly different from that of the Ghanaian population. This supports conservation efforts in the southern African region. High levels of inbreeding in both populations are troubling, and continued genetic monitoring of both populations should take place to ensure that current genetic diversity is maintained.

Chapter 4

Family associations in a breeding colony of Hooded Vulture (*Necrosyrtes monachus*) in the Lowveld of South Africa

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Abstract

Vultures across Africa have experienced recent population declines, and Hooded vultures (*Necrosyrtes monachus* Temminck, 1823) are no exception with populations showing severe declines across most of their distribution. The range-edge population in South Africa is one of the smallest populations, with an estimated size of only 100-200 individuals and it is of conservation concern. This species' egg-laying starts in early June and tends to end around August. They commonly nest in jackal-berry trees (*Diospyros mespiliformis*) and common cluster figs (*Ficus sycomorus*) close to flowing water. Hooded vultures have relatively small home ranges compared to other larger vulture species in Africa. Communal roosting sites function as information sharing hubs, a phenomenon not seen in Hooded vultures as such, but has been seen in Cape vultures (*Gyps coprotheres*), and other cliff-nesting raptor species. Naturally moulted feathers (n = 108) were collected below nests, and 14 microsatellite loci markers were used for genetic analyses. Using genetic data, we examined the relatedness between birds at particular nests to see if more related individuals breed closer together and thus function as a food finding information sharing hub. Using the genetic data, we also examine relatedness and nest turnover of nesting individuals along the Olifants River and other locations in the Lowveld by sampling well-established nests over five consecutive years. Mantel tests were done for both relatedness coefficient estimators TrioML ($r = 0.032$, $R^2 = 0.001$, $p = 0.224$), and LynchRD ($r = 0.007$, $R^2 = 0.00005$, $p = 0.403$) compared to geographical distance and showed no statistical correlation. The Mantel test done with Nei's genetic distance did show a negative correlation ($r = -0.108$, $R^2 = 0.0117$, p-value = 0.012), indicating that individuals that were more closely related tended to breed further away from each other. The spatial autocorrelation analysis did indicate some positive correlation for some distance classes; however, the majority of these distance classes showed a negative correlation. This suggests that individuals are less related than expected by chance for certain distances between nests. The Wilcoxon test indicated a significant difference between the mean relatedness for individuals breeding along the Olifants River compared to individuals breeding away from the Olifants River for the LynchRD relatedness coefficient estimator ($p = 0.000001$). Our results

did not support the hypothesis that these loose colonies function as food finding information sharing hubs. No nests were reused by individuals in the Lowveld area of South Africa. However, the Olifants River should be considered as an important breeding site for this species, and the conservation of nesting trees in this area should be prioritised.

Key words: Hooded vulture, South Africa, Olifants River, *Necrosyrtes monachus,* Nest use

Introduction

The Hooded vulture (*Necrosyrtes monachus* Temminck, 1823), is widespread in sub-Saharan Africa (eBird, 2021; Ferguson-Lees and Christie, 2001). Although widely distributed, locally the species is resident and generally sedentary, with some limited dispersal by non-breeders and immature birds (Ferguson-Lees and Christie, 2001; Reading et al., 2019; Thompson et al., 2020). The species has experienced population declines across most of its distribution as a result of anthropogenic factors such as poisoning (intentional and accidental), collisions with pylons and powerlines, and poaching for use in traditional medicine (Odino et al., 2014; Ogada et al., 2012; Ogada and Buij, 2011; Saidu and Buij, 2018; Williams et al., 2021). This species is currently listed as Critically Endangered by the IUCN (BirdLife International, 2022).

Although large flocks of Hooded vultures are common in West Africa (Gbogbo and Awotwe-Pratt, 2008; Jallow et al., 2022, 2016; McLachlan and Liversidge, 2016), the range-edge South African population is much smaller, including only 100-200 mature individuals (Taylor et al., 2015). The conservation value of these small peripheral populations is debatable, as smaller populations tend to lose genetic diversity through the processes of genetic drift and inbreeding. On the other hand, these range-edge populations may act as important reservoirs of rare alleles and may provide important information on how the species may react under various climate change scenarios (Eckert et al., 2008; Nowell et al., 2022; Sagarin and Gaines, 2002). In the 1980s, the breeding population of South Africa was estimated to be lower than 50 pairs, but in the early 2000s, the breeding populations increased to around 50-100 pairs; this increase in breeding pair numbers could be a result of more research into the species rather than a direct indication of an increase in population size for southern Africa (Daneel, 1984; Monadjem et al., 2016; Roche, 2006). Understanding the dynamics of this South African population of Hooded vultures could provide important information for the conservation of the species.

Communal roosts or breeding colonies of scavengers such as vultures may act as "food finding information hubs" allowing for the exchange of information regarding potential food sources

(Dermody et al., 2011; Ward and Zahavi, 1973). This may in part explain the high levels of relatedness often found within colonies (Erwin, 1978; Waltz, 1982), with close relatives more likely to tolerate the cost of sharing food by increasing their inclusive fitness (Hamilton, 1964). Although not observed in Hooded vultures, indirect evidence in other vulture species such as the Cape vulture (*Gyps coprotheres*) suggests that communal roosts or colonies do act as important food finding centres, where individuals communicate sources of potential food and transfer other information (Dermody et al., 2011; Martens et al., 2020).

Hooded vultures are monogamous and may use the same nest all year round. Unlike other tree-nesting vulture species that generally nest on the tops of trees, Hooded vultures usually nest within the canopies of well-foliaged trees such as jackal-berry trees (*Diospyros mespiliformis*) and common cluster figs (*Ficus sycomorus*) along water courses (Fern et al., 2022; Monadjem et al., 2016; Mundy et al., 1992; Roche, 2006); this makes direct observation of nesting behaviour difficult. Nest-site selection could be adaptive in the species as nests often remain active for many years. For example, one of the first breeding records available for the Kruger National Park (South Africa) was a nest in Bangu Gorge recorded in 1967, and this same nest was still active twenty years later (Daneel, 1984; Monadjem et al., 2016; Roche, 2006). It is unclear whether a pair of vultures uses the same nest throughout their life and whether offspring will take over their parents' nest.

Unfortunately, there is limited information available on many aspects of Hooded vulture life history including the factors affecting reproduction in the wild (Bamford et al., 2009; Pfeiffer et al., 2017; Plaza et al., 2020). An improved understanding of the population dynamics of the South African population could benefit conservation efforts (Carrete et al., 2006; Margalida et al., 2008; Reading et al., 2005; Zuberogoitia et al., 2008). For example, an increased understanding of nest fidelity in this species could help guide whether nesting trees/habitats need to be protected (Thompson and Blackmore, 2020). Also, if Hooded vulture colonies are operating as "food finding information hubs", then it is predicted that individuals nesting close to each other should be more closely related and that individuals should use the same nests over multiple years. These predictions were tested in this study by determining the relatedness of individuals nestling along a section of the Olifants River in Limpopo, South Africa, using genetic data from fourteen microsatellites. Samples collected from the same nest over many years were used to determine if the same individuals were using the same nest over multiple years.

Materials and methods

Sampling

Most sampling took place along a 94.06 km section along the Olifants River, South Africa. This transect was chosen as the Lowveld (including Limpopo and Mpumalanga provinces) is the core distribution for this species in South Africa, with the Olifants River being some of the prime habitat for nesting. When possible, all nests that were found along the transect were sampled. Opportunistic sampling was done when nests were located in different areas in the Lowveld, with two nests sampled 67.84 km and 163.67 km away from the core distribution along the Olifants River. A total of 49 Hooded vultures' nests were sampled (Figure 4.1). Moulted feathers were collected underneath and inside of nests during breeding seasons between 2015-2019. A total of 108 moulted feather samples were collected. A total of nine nests samples were collected over multiple years. This subset of the data (n = 42) was used to assess nest turnover and determine if the same individuals were using the same nest over multiple years.

Ethical clearance for this project was granted by the Animal Research Ethics Committee of the University of Kwazulu-Natal (AREC/094/015PD, AREC/022/020). A Section 20 permit (ref 12/11/1/5; 2104NC) and a veterinary permit (ref MJV GR 76/22) were obtained for the collection and movement of samples. Fieldwork was done with provincial research permits for Limpopo Province (ZA/LP/HO/2937 (Oct 2015 - Oct 2016), ZA/LP/80214 (Jan 2017 to Jan 2018), ZA/LP/90993 (Aug 2018 to Aug 2019), ZA/LP/100606 (Oct 2019 to Oct 2020)), and for Mpumalanga Province (permit no. MPB. 5557 (in 2016), 5581 (in 2017), 5619 (in 2018)).

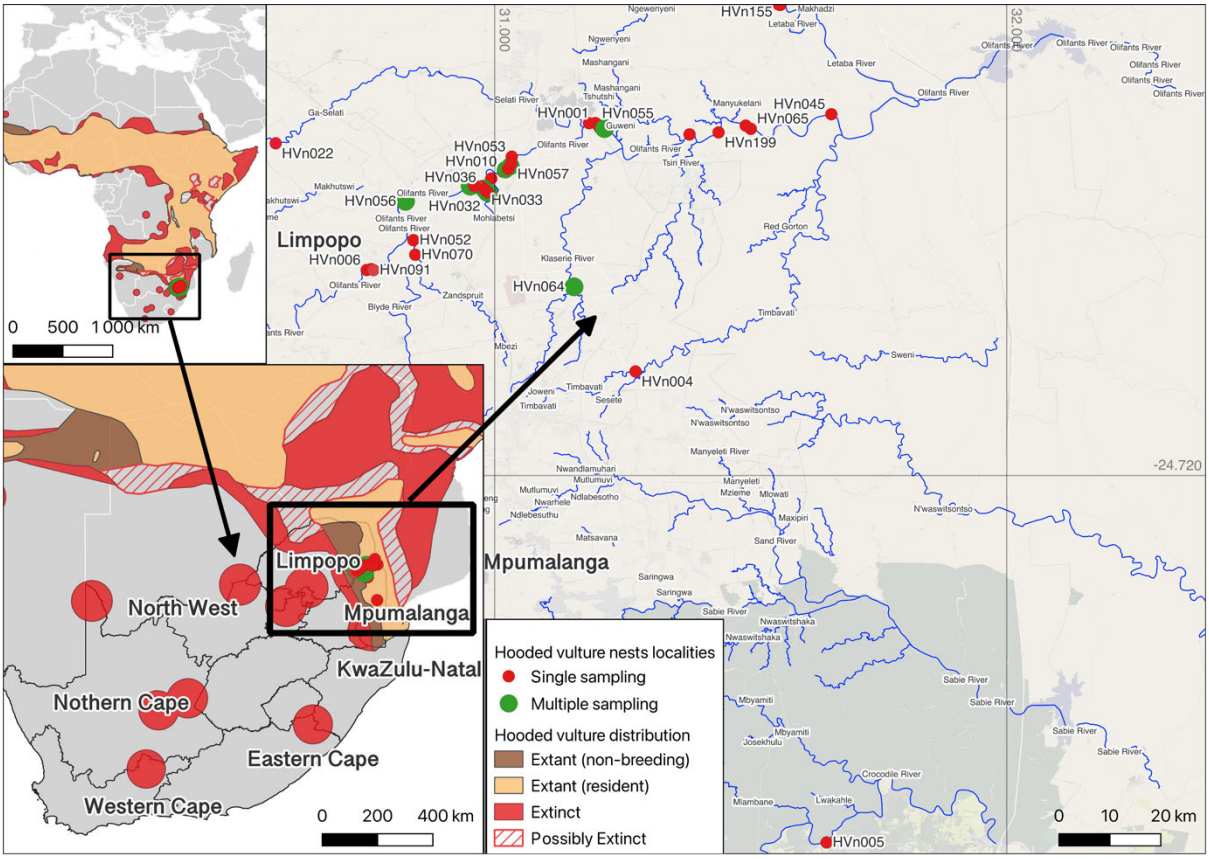

Figure 4.1: The localities of Hooded vulture (*Necrosyrtes monachus)* nests in the north-eastern parts of southern Africa. Nests were sampled between 2015-2019. The nests that were sampled once are shown by the red dots (lable indicating the given nest label when sampled). The nests that were sampled over multiple years are indicated in green.

DNA extraction and microsatellite amplification

The E.Z.N.A® Tissue DNA kit (Omega Bio-Tek, United States) was used for all DNA extractions. DNA was extracted from the calamus of the feathers. Small modifications were made to the extraction protocol to ensure sufficient DNA yield from feathers. The modifications include incubating samples in proteinase K and lysis buffer for 24 hours in a heated water bath (56°C). For the first five hours the samples were removed every hour to be vortexed and were then placed back into the water bath. The lysate was incubated in B3 buffer for 45 minutes (70°C), the final volume of pre-warmed Buffer BE was added to the spin column, centrifuged, then reheated for 5 minutes and reapplied to the membrane before a final centrifuge step. All DNA extracts were stored at -20°C.

Fourteen microsatellite loci (Table 4.1) were used to calculate the relatedness of individual Hooded vultures in this study. These microsatellite loci were used in previous studies of *Gyps*

coprotheres, *Gypaetus barbatus* (Kleinhans and Willows-Munro, 2019; Streicher et al., 2021) and other vulture species (Çakmak et al., 2019; Davidović et al., 2020). The loci were amplified in five multiplex reactions using the Multiplex TEMPase PCR Kit (AMPLIQON, Denmark). Each forward primer was fluorescently labelled. The details of primers used in each multiplex are provided in Table 4.1.

Each 10 µl reaction consisted of ~2-30 ng of DNA template, 5 µl of Multiplex TEMPase mix and 0.2 µl of 0.2 µM for each fluorescently tagged primer, and purified water. The thermocycler parameters were as follows: initial denature at 94°C for 15 min, then followed by 94°C for denaturing, 60°C for annealing and then 72°C for elongation for a total of 35 times. Then a final elongation at 72°C for 10 min and an hold at 4°C until samples were removed from the thermocycler. Negative controls were included in all sets of reactions. All amplified products were sent to the Central Analytic Facility at Stellenbosch University (South Africa) for fragment analyses. Genotype scoring was performed using GeneMarker v2.6.3 (Soft Genetics). To ensure genotype consistency, all samples were re-amplified at least seven times and checked for consistency.

Table 4.1: List of loci used in genetic analyses of South African Hooded vulture (*Necrosyrtes monachus*), primers used for amplification, fluorescent dyes used for tagging the forward primers, annealing temperature (Tm, °C) used in each reaction for the amplification of specific microsatellite loci with the combination of loci amplified together in multiplex reactions.

Loci Name	Primer pair	Dye	Tm	Motif	Multiplex
BV2	F: CAGCATGTTATTTTGGCTGC	HEX	60	CA_{11}	A
	R: TTGCTAAACCGGTTAGAAGTTG				
BV6	F: AATCTGCATCCCAGTTCTGC	HEX	60	CA_{11}	D
	R: CCGGAGACTCTCAGAACTTAAC				
BV8	F: TGGCATGCTGCTATGAGAAC	FAM	60	CA_{11}	C
	R: GTGCTTTGCATGCTTTTACTC				
BV9	F: ATCTAGGGACATCGAGGAGC	HEX	60	TA_6 CA_{11}	D

	R: ACAGGGATGCAGGTAAGCC				
BV11	F: TGTTTGCAAGCTGGAGACC	HEX	60	CA_{22}	B
	R: AAAAGCCTTGGGGTAAGCAC				
BV12	F: TCAGGTTTTGACGACCTTCC	FAM	60	CA_{15}	C
	R: GTGGTAACGGAGGAACAAGC				
BV13	F: AAAACAGAGTTTTCACATTTTCATAAG	FAM	60	CA_{16}	A
	R: TTCAGGAAACAGAAGCATGAAC				
BV17	F: TGATGTGCAGATGCGTGAC	HEX	60	CA_{11}	C
	R: GGACTCTGATGAAGCCAAGC				
BV20	F: GAACAGCACTGAACGTGAGC	HEX	58	CA_{13}	E
	R: GTTTCTCCTGACAGTGAAATAACTC				
Gf3f3	F: GATCTTTCCCCTTCTGTG	TET	60	CT_{10}	E
	R: TTCGTGCAGTGATGCTGGTG				
Gf3H3	F: GTAGAATAATTTGCTCCTGG	FAM	60	CT_{12}	D
	R: GTGAAGGCACCTCATAGACA				
Gf8G	F: TGAGCAGGTGAGTCCAGAAG	FAM	60	$CT_8C\ TC_2$	B
	R: GCTCTCCTGTCATCTTGCAT				
Gf9C	F: GGTGGACATTACATACACTG	HEX	60	TC_{10}+$CT_9\ C$ $CA_5T\ AC_4$	A
	R: CAAGGAATCTGGACTACTAA				
Gf11A4	F: GATCCCTTCCAACCGAAAAT	HEX	60	$CTCTT_{17}$	C
	R: TGGTGACCAACGGAAGTGTG				

Data analyses

For basic genetic diversity assessment, an identification test was done using Cervus v3.0.752 (Kalinowski et al., 2007) to ensure duplicate samples were not used. Cervus was also used to

estimate polymorphic information content (PIC) for each locus to ensure that loci were variable enough for the analyses proposed. Null allele frequencies were estimated using FreeNA (Chapuis and Estoup, 2007). GenALEx v6.502 (Peakall and Smouse, 2006) was used to assess the number of alleles (A) per loci and the number of effective alleles (N_e). Universal Transvers Mercator grid data for each nest was obtained in QGIS v3.26 (QGIS Development Team, 2021) then the geographical distance matrix between nests was calculated in GenALEx v6.502 (Peakall and Smouse, 2006).

Relatedness of individuals across the landscape

The relatedness of Hooded vulture individuals was estimated using the program Coancestry v1.0.1.9 (Wang, 2011). This program calculates relatedness using a range of different methods (Wang, 2011). The internal individual relatedness coefficient was calculated using Lynch and Ritland (1999; LynchRD), and the triadic maximum likelihood (TrioML; Wang 2007). The Lynch and Ritland test calculates relatedness based on the probability of alleles of a random locus being identical by descent (Lynch and Ritland, 1999; Wang, 2011). The Triadic maximum likelihood method uses a maximum likelihood to calculate the relatedness between two individuals for a set of population allele frequencies (Wang, 2007). These analyses were conducted without first-order kin accounting for inbreeding and an error rate of 0.0005. Nei's genetic distance between individuals collected at nests was also calculated in GenALEx v6.502. This measure is the number of gene differences per locus taking into account the polymorphism of locus within a population (Nei 1972).

A Mantel test (Mantel, 1967) was done to assess if there is a significant correlation between relatedness using LynchRD and TrioML, and geographical distance. These tests were performed in GenALExv6.502 (Peakall and Smouse, 2006). Spatial autocorrelation was also conducted in GenALEx v6.502, using 100 even distance classes of 2 km each (the closest distance between two nests), for 1000 permutations and 1000 bootstrap replicates of pairwise comparisons within each class. Bootstraps provided indications of a 95% confidence interval within each distance class.

To further assess the fine scale population structure between individuals and their nest site location, the relatedness between the individuals breeding along the Olifants River and away from the Olifants River was compared. This was done by comparing the mean relatedness between the two by implementing a non-parametric two-sample Wilcoxon test in R v4.2.1 (2022-06-23) (R Core Team, 2022). The Wilcoxon test is a statistical test that measures the

difference between the mean of two given parameters for a set of two populations. A 95% confidence level was applied for all tests.

To further visualise the genetic relationships between individuals, allele sharing networks were created using EDENetworks v2.18 (Kivelä et al., 2015). In these networks, individuals are represented as nodes with connections between nodes weighted by their pairwise genetic distance (Fst).

Results

Fourteen microsatellite markers were successfully amplified from 108 Hooded vulture (*Necrosyrtes monachus*) feathers, collected below and inside the nests found in the Lowveld region of South Africa. The information value of these loci (Table 4.2) for the South African population indicates that eleven of the loci (BV2, BV13, Gf9C, BV11, BV12, Gf11A4, BV9, BV6, Gf3H3, BV20, Gf3f3) were highly informative (PIC > 0.5). Six of the loci (Gf8G, BV8, Gf11A4, BV9, BV20, Gf3f3) showed elevated levels of null alleles ($N_0 > 0.12$). The Lowveld Hooded vulture population showed a number of alleles per locus (A) ranging from 4.000 - 18.000 with a mean effective number of alleles (N_e) of 3.442, with five loci having $N_e > 4$ (Gf9C, BV12, Gf11A4, BV6, Gf3H3).

Table 4.2: Summary showing the statistics of Hooded vulture (*Necrosyrtes monachus*) from South Africa sampled in the Lowveld area. It shows the number of alleles (A), number of effective alleles (N_e), null allele frequency (N_o), and the polymorphic information content (PIC) of each locus.

Locus	A	N_e	N_0	PIC
BV2	4.000	2.383	0.118	0.531
BV13	10.000	2.458	0.042	0.552
Gf9C	17.000	5.717	0.069	0.804
BV11	12.000	2.730	0.021	0.586
Gf8G	6.000	1.505	0.150	0.317
BV17	8.000	1.228	0.089	0.182

BV8	4.000	1.441	0.241	0.279
BV12	17.000	6.227	0.063	0.822
Gf11A4	9.000	4.902	0.292	0.767
BV9	9.000	2.944	0.155	0.621
BV6	18.000	6.340	0.065	0.825
Gf3H3	12.000	4.172	0.000	0.725
BV20	8.000	2.261	0.134	0.525
Gf3f3	6.000	3.881	0.145	0.699
Mean	10.000	3.442	0.113	-

Nest usage over time

Despite previous studies indicating that South African Hooded vultures are resident and generally sedentary (Ferguson-Lees and Christie, 2001), we were not able to resample the same individuals at the same nest. Cervus did not detect any duplicate individuals when testing the nests that were resampled multiple times. Therefore, no genetic evidence seen indicating that the same individuals used the same nest over multiple years. Therefore, this species might be sedentary within the area but moves frequently to find more suitable breeding habitat.

Relatedness of individuals across the landscape

Most individuals showed a genetic distance estimation between 0.100 – 0.500, for Nei's genetic distance estimator as seen in the Mantel test (Figure 4.4 A). The Mantel test also showed a significant negative correlation between geographical distance and genetic distance r = - 0.108, R^2 = 0.0117 (p = 0.012), for 9 999 permutations for Nei's genetic distance estimator (Figure 4.4 A). The negative correlation indicated that the further away individuals breed from each other, the shorter the genetic distance is between individuals. However, the Mantel test performed using the TrioML relatedness coefficient between individuals (Figure 4.2 B) indicated no correlation between the TrioML relatedness coefficient and geographical distance (r = 0.031, R^2 = 0.001, p-value = 0.229). The same results were seen for the Mantel test to assess the LynchRD relatedness coefficient with geographical distance (Figure 4.2 C), where the Mantel

test also did not indicate any correlation between the LynchRD relatedness coefficient and geographical distance (r = 0.007, R^2 = 0.0005, p-value = 0.409).

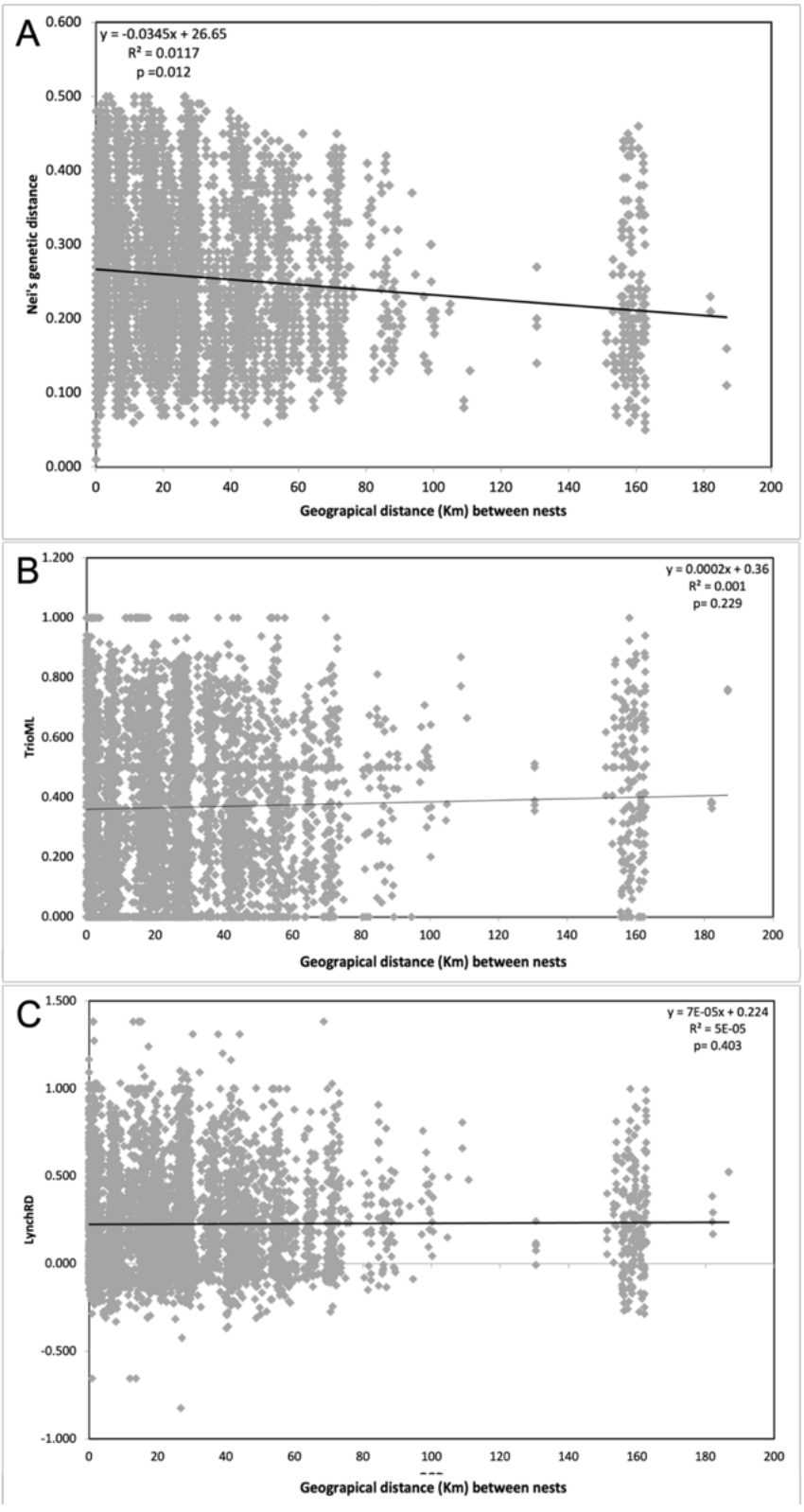

Figure 4.2: Mantel test plotting (A) Nei's genetic distance (y-axis), (B) TrioML and (C) LynchRD relatedness coefficient against geographical distance (km) between nests (x-axis),

for the Hooded vulture (*Necrosyrtes monachus*) feathers (n = 108) from South Africa's Lowveld area.

The spatial autocorrelation analysis indicated a significant correlation between the genetic distance and the geographical distance for some distance classes. These classes included distance class 2-6 km, 10-18 km, 38-42 km, 50-58 km, 98-102 km, 110km-114 km, 150-158 km and then final 185-190 km (Figure 4.3). This indicates that individuals in these classes were genetically more similar than expected by chance. Some distance classes indicated a negative correlation (30-34 km, 46-50 km, 74-78 km, 78-86 km, 90-94 km), indicating that individuals in these classes were genetically less similar than expected by chance (Figure 4.3). This graph indicates that the population is less genetically similar than expected by chance for most of their distribution (Figure 4.3). Thus, indicating that nest selection is more random in this species than suspected, and could be based more on suitable breeding habitats than relatedness between individuals.

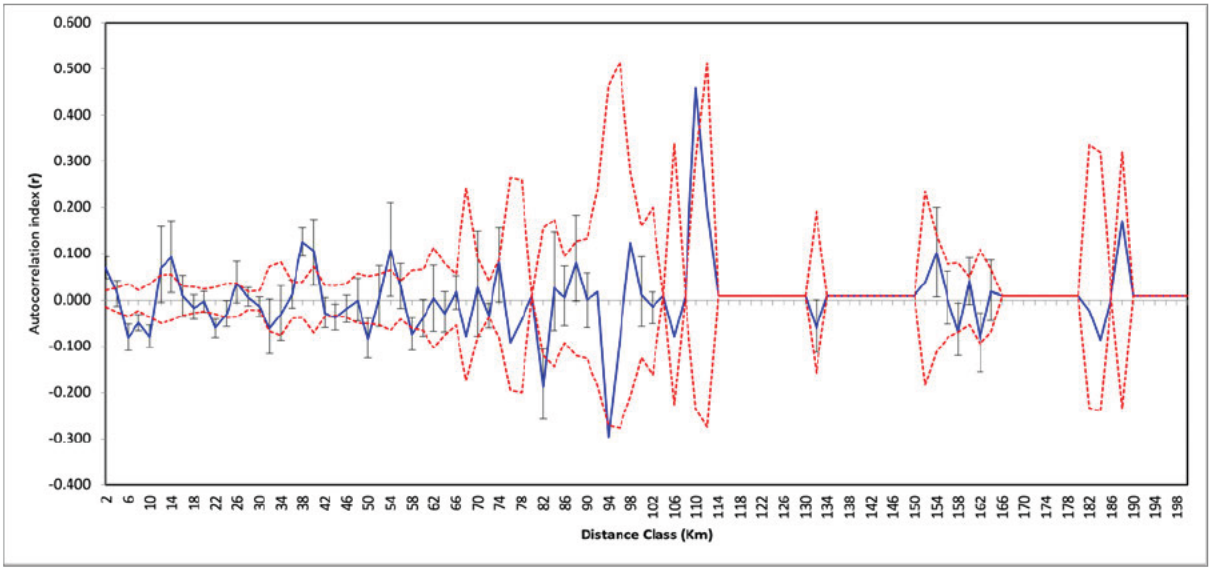

Figure 4.3: The spatial autocorrelation analysis done for our samples of Lowveld Hooded vulture (*Necrosyrtes monachus*) population. The solid blue line indicates the autocorrelation coefficient data with a 95% confidence interval indicated by the black error bars, and the dotted red line indicates the 95% confidence interval around the null hypothesis.

The Wilcoxon test did not indicate a significant difference between the mean relatedness for TrioML relatedness measures for individuals breeding along the Olifants River compared to

individuals breeding away from the Olifants River (p – value = 0.069) system (but close to another river system) (Figure 4.4 A). The Wilcoxon test did indicate a significant difference in the mean relatedness between individuals breeding away from the Olifants River and individuals breeding along the Olifants River (p - value = 0.00000016) for the LynchRD relatedness coefficient (Figure 4.4 B).

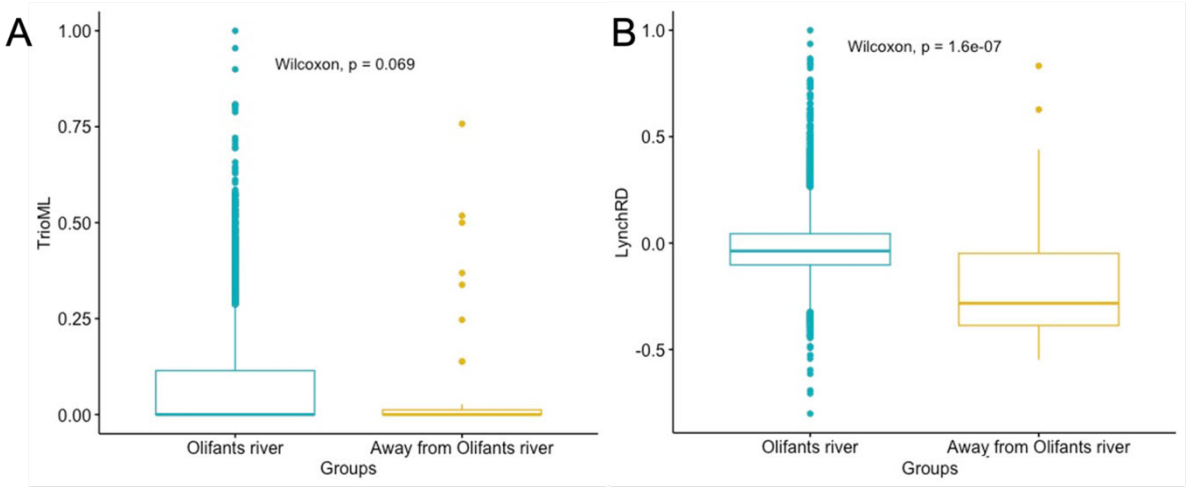

Figure 4.4: Boxplots indicating the relatedness between Hooded vulture (*Necrosyrtes monachus*) individuals breeding along the Olifants River and away from the Olifants River for relatedness coefficient estimator TrioML (A), and LynchRD (B). The text insert indicates the p-value of the two-sample Wilcoxon test, for a difference in means.

Looking at the relatedness network between individuals (Figure 4.5), it is clear that all individuals share alleles. It can be seen that some nodes (individuals) share more alleles with other nodes; this is indicated by the size of the node. Individuals breeding along the Olifants River share more allelic similarities with individuals in the surrounding area than with individuals breeding further away from this river.

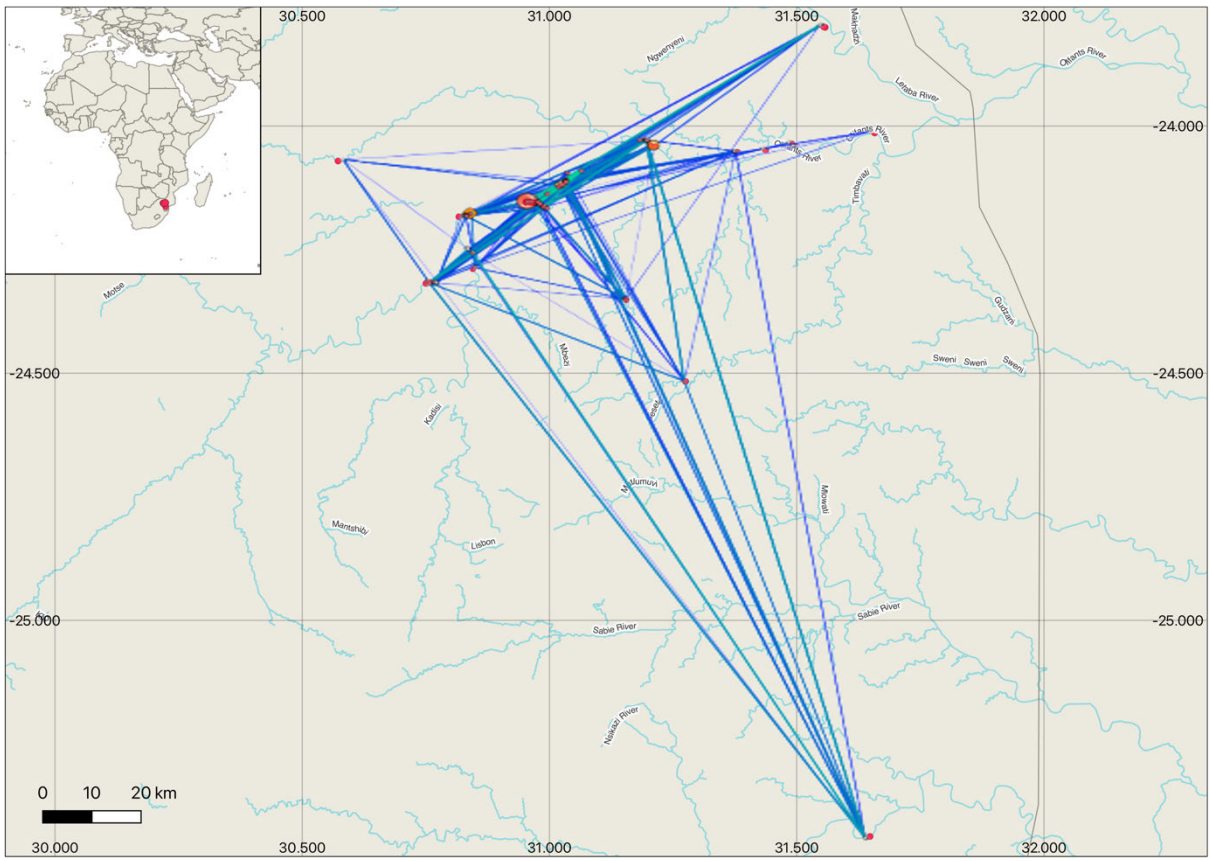

Figure 4.5: Locations of some Hooded vulture (*Necrosyrtes monachus*) nests in the north-eastern part of southern Africa. The relatedness network (constructed using EDENetworks) has been overlayed.

Discussion

The breeding success of the Hooded vulture is of conservation concern because of the small size of the population in Southern Africa. Despite previous studies noting that Hooded vultures in southern Africa are resident and generally sedentary (Ferguson-Lees and Christie, 2001; Reading et al., 2019; Thompson et al., 2020) in this study no individuals were found to reuse nests over a sampling period of 5 years. Thus, this species is fairly sedentary within the area, but frequently moves to find better breeding habitat. This could be based on the success of a specific nesting area or how often the nest is visited by other species that could interfere with breeding. A similar study conducted on White-tailed eagles (*Haliaeetus albicilla*) showed that these birds of prey often use alternative nests for breeding (Bulut et al., 2016). This could be a similar case for Hooded vultures, where alternative nests are used in different breeding seasons in different areas.

However, the high density of Hooded vulture nests found along the Olifants River, and reduced levels of relatedness and higher levels of shared alleles between individuals shown in the EDENetworks could be due to the habitat being more suitable for breeding. This could also indicate high competition for nesting within this area. The high density of nests in this area has previously been reported with nests being as close as 0.76 km from one another. In northern Zimbabwe, nearest-neighbour distances for Hooded Vulture nests were as low as 50 m (Monadjem et al., 2016; Mundy, 1982). These high nest densities could result from the high density of preferred nesting trees that provide appropriate cover for Hooded vulture nests (Monadjem et al., 2016; Mundy, 1982; Roche, 2006).

Higher levels of relatedness between individuals within a colony could increase the tolerance between individuals to act as a food finding information sharing hub (Dermody et al., 2011; Erwin, 1978; Waltz, 1982; Ward and Zahavi, 1973). This is due to the fact that this increases the inclusive fitness of individuals within the colony (Hamilton, 1964). However, the negative correlation showed by the Mantel test indicates that nesting sites around the Olifants River may not be a communal breeding sites for Hooded vultures that serves as a food finding comunication hub between related individuals. This type of behaviour where breeding colonies are formed is seen in White-backed vultures (*Gyps africanus*), where nests occur in colonies rather than single nesting sites (Johnson and Murn, 2023). These colony nesting sites in White-backed vultures showed positive effects on the breeding success of these birds (Johnson and Murn, 2023). Similar observations have been made in Griffon vultures (*Gyps fulvus*), where informed individuals are followed to food resources by uninformed individuals (Harel et al., 2017)

My study showed that there is no evidence supporting the hypothesis that individuals nesting along the Olifants River function as a food finding information sharing hub, due to increased levels of relatedness between individuals. However more ecological studies need to be done to determine if they function as a food finding information sharing hub due to other factors. Similarly, and contrary to expectations, this study was not able to detect any individuals that reused nests over a five-year period, as seen in other raptor species such as booted eagle (*Aquila pennata*) and common buzzard (*Buteo buteo*; Jiménez-Franco et al., 2014). However, this study was able to show that the Olifants River is an important breeding site for the Lowveld Hooded vulture population.

Chapter 5

Conclusion and recommendations

Populations of Hooded vultures (*Necrosyrtes monachus*, Temminck, 1823) have experienced accelerated declines over the last century due to anthropogenic factors, such as human persecution, hunting for traditional medicine, and intentional and unintentional poisoning. This is a concern as Hooded vultures (along with other vultures and scavengers) are important in ecosystem health and likely in disease control too. This MSc study had a number of key aims. First, to clarify the taxonomic status of the two Hooded vulture subspecies (*Necrosyrtes monachus monachus* Temminck, 1823, *Necrosytres monachus pileatus* Burchell, 1824) and examine the genetic diversity of the species across their pan-African distribution using a set of microsatellite loci (Chapter 2). A second aim was to assess the overall genetic health and diversity of the range-edge population found in South Africa and to do a comparison study to a population located in the centre of the species' distribution (Chapter 3). This study also aimed to provide important insight into the population dynamics of Hooded vultures in South Africa and examined processes such as nest use, dispersal and natal philopatry of the southern African population found in the greater Limpopo area (Chapter 4).

Currently Hooded vultures are split into two subspecies: *Necrosyrtes monachus monachus*, that inhabits the North and western parts of Africa, and *Necrosyrtes monachus pileatus* that inhabits the South and eastern parts of Africa. The two subspecies differ in tolerance to urbanization with the *N. m. monachus* subspecies comfortable in urban environments and often found feeding at landfill sites and along the beach, while *N. m. pileatus*, being less commensal, occurs predominantly in protected areas (Barlow et al., 2022; Barlow and Brohaugh, 2022; Mikkola and Barlow, 2022) The two subspecies are distinguishable by subtle differences in body size, although previous authors have argued that this characteristic is due to size cline rather than a defining characteristic (Mundy, 2021). The validity of this subspecies taxonomic nomenclature has been debated for several years. Many scientists believe that this group should be classified as monotypic, and that the differences seen between the two groups is size cline and not speciation.

In this study we use genetic data to test this hypothesis. The data presented in this thesis does not support the delimitation of two subspecies. Instead, we suggest that Hooded vultures be considered a single monotypic species. I did, however, find that populations in different portions of distribution have undergone quite different demographic events. Populations in

West Africa and East and southern Africa experienced bottlenecks in response to the expansion of the central African forests. For the southern and East African populations these bottleneck events were around 77 000 years ago when the forest of central Africa was much larger due to a moister climate. For the West African populations this bottleneck event was more recent, at around 7 000 years ago. This corresponds with the central African forest expansion and the Bantu migration. Thus, we also conclude that region-specific environmental management plans should be implemented in different portions of the species' distribution as individual populations are facing declines due to contrasting anthropogenic and natural factors. Therefore the species may not survive under a one-fits-all type of environmental management plan.

The range-edge Hooded vulture population found in South Africa is small. The importance of these range-edge populations to the genetic diversity and survival of species has been discussed to great lengths in the scientific literature. There are two main schools of thought, first that range-edge populations are small and through the process of genetic drift and inbreeding could be genetically depauperate. If this were true, then these range-edge populations should not be a conservation priority. In contrast, range-edge populations may be reservoirs of important genetic diversity. Studying range-edge populations can provide important information on how species will adapt to environmental change, which is particularly important in light of climate change. In Chapter 3 of this thesis, I compared the genetic diversity present in the range-edge South African population to that of a population in Ghana. I found that the genetic diversity of these two populations is very similar and that both the Ghanaian and South African populations show signs of inbreeding and high levels of relatedness. The South African and Ghana populations show no signs of recent bottleneck events. The South African and Ghana populations showed signs of ancient bottleneck events that occurred in the last 100 generations. Using Approximate Bayesian Computation (ABC), recent expansion events related to human expiation and savanna biome expiation were seen in both populations.

In South Africa Hooded vultures' nests are largely confined to conservation areas, and understanding their breeding ecology would allow for better conservation management decisions. In vulture species such as Griffon vultures (*Gyps fulvus*) it has been shown that breeding colonies function as food finding information sharing hubs. It has also been shown that vultures within the Griffon vulture colonies have higher levels of relatedness and that one individual can share up to three kin matches. Hooded vultures have very small home ranges when compared to larger vulture species and are usually sedentary, therefore the general assumption was that nests were reused by individuals to reduce the energy cost needed to

construct new nests each season. In this study I studied whether if there was a correlation between geographic distance between Hooded vulture nests and relatedness along a 94.06 km transect along the Olifants River, South Africa. Although my study did highlight the riparian zone along the Olifants River as key breeding location for this species, it does not support the hypothesis that individuals that nest close together are closely related. Nest competition around the Olifants River could be higher than in other areas due to availability of suitable nesting trees. This study did not find any evidence of nest reuse in the area. This surprising finding should be examined further using telemetry.

The results from this study will contribute towards the conservation of the Hooded vulture. This study should also be considered as a steppingstone for further research on this population. This study shows the need for GPS and telemetry studies on this species to better understand the movement between each country, and the need to assess the movement within the confinements of the conservation areas of southern Africa. Both this study and further GPS and telemetry studies should be used in the formation in conservation management plans, to ensure the survival of this species. These management plans should incorporate the main goals as set out by the Vulture Multi-species action plan. However, the main management plans for each country would need to be assessed separately, as different factors affect different populations. In most countries however the need for captive breeding programs with native birds are needed to ensure that genetic outbreeding and inbreeding does not occur. Thus, this study can be seen as a base line to assess the different genetic groups to which individuals can be used for breeding programs. The need for habitat resotoration is needed for most countries as well due to a lack of appropriate nesting sites. In some countries the need for human conflict mitigation is needed to ensure that the species is not prosecuted by locals.

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Appendix

Appendix 2.1 Table showing the country in which the Hooded vulture (*Necrosyrtes monachus*) sample was collected, the type of sample that was collected and the GPS coordinates of that sample. Then finally where applicable the museum the sample was sourced from.

ID	Country collected	Subspecies	Sample type	GPS coordinates		**Source**
				Latitude	Longitude	
NME01	ETHIOPIA	*N. m. pileatus*	FEATHER	9.1450	40.4897	Evan R. Buechley
NME02	ETHIOPIA	*N. m. pileatus*	FEATHER	9.1450	40.4897	Evan R. Buechley
NME03	ETHIOPIA	*N. m. pileatus*	FEATHER	9.1450	40.4897	Evan R. Buechley
NME04	ETHIOPIA	*N. m. pileatus*	FEATHER	9.1450	40.4897	Evan R. Buechley
NME05	ETHIOPIA	*N. m. pileatus*	FEATHER	9.1450	40.4897	Evan R. Buechley
NME06	ETHIOPIA	*N. m. pileatus*	FEATHER	9.1450	40.4897	Evan R. Buechley
NME07	ETHIOPIA	*N. m. pileatus*	FEATHER	9.1450	40.4897	Evan R. Buechley
NME08	ETHIOPIA	*N. m. pileatus*	FEATHER	9.1450	40.4897	Evan R. Buechley
NME09	ETHIOPIA	*N. m. pileatus*	FEATHER	9.1450	40.4897	Evan R. Buechley
NM34	SOUTH AFRICA	*N. m. pileatus*	FEATHER	-24.1840	30.8260	L.J. Thompson
NM11	SOUTH AFRICA	*N. m. pileatus*	FEATHER	-24.2885	30.8449	L.J. Thompson
NM12	SOUTH AFRICA	*N. m. pileatus*	FEATHER	-24.2885	30.8449	L.J. Thompson
NM13	SOUTH AFRICA	*N. m. pileatus*	FEATHER	-24.1206	31.0265	L.J. Thompson
NM14	SOUTH AFRICA	*N. m. pileatus*	FEATHER	-24.3512	31.1549	L.J. Thompson
NM15	SOUTH AFRICA	*N. m. pileatus*	FEATHER	-24.1665	30.9857	L.J. Thompson

NM16	SOUTH AFRICA	*N. m. pileatus*	FEATHER	-24.1593	30.9786	L.J. Thompson
NM17	SOUTH AFRICA	*N. m. pileatus*	FEATHER	-24.1224	31.0209	L.J. Thompson
NM18	SOUTH AFRICA	*N. m. pileatus*	FEATHER	-24.1151	31.0311	L.J. Thompson
NM19	SOUTH AFRICA	*N. m. pileatus*	FEATHER	-24.1151	31.0311	L.J. Thompson
NM20	SOUTH AFRICA	*N. m. pileatus*	FEATHER	-24.0533	31.3807	L.J. Thompson
SH1	SENEGAL	*N. m. monachus*	FEATHER	14.4974	-14.4524	S. Willows-Munro
SH2	SENEGAL	*N. m. monachus*	FEATHER	14.4974	-14.4524	S. Willows-Munro
SH3	SENEGAL	*N. m. monachus*	FEATHER	14.4974	-14.4524	S. Willows-Munro
SH4	SENEGAL	*N. m. monachus*	FEATHER	14.4974	-14.4524	S. Willows-Munro
SH5	SENEGAL	*N. m. monachus*	FEATHER	14.4974	-14.4524	S. Willows-Munro
SH6	SENEGAL	*N. m. monachus*	FEATHER	14.4974	-14.4524	S. Willows-Munro
SH7	SENEGAL	*N. m. monachus*	FEATHER	14.4974	-14.4524	S. Willows-Munro
SH8	SENEGAL	*N. m. monachus*	FEATHER	14.4974	-14.4524	S. Willows-Munro
SH9	SENEGAL	*N. m. monachus*	FEATHER	14.4974	-14.4524	S. Willows-Munro
GHV18	GHANA	*N. m. monachus*	FEATHER	7.1403	0.4749	J. Deikumah
GHV22	GHANA	*N. m. monachus*	FEATHER	7.1453	0.4774	J. Deikumah

GHV59	GHANA	*N. m. monachus*	FEATHER	7.1564	0.4958	J. Deikumah
GHV31	GHANA	*N. m. monachus*	FEATHER	7.1425	0.4765	J. Deikumah
GHV46	GHANA	*N. m. monachus*	FEATHER	5.1284	-1.2954	J. Deikumah
GHV1	GHANA	*N. m. monachus*	FEATHER	5.1288	-1.2913	J. Deikumah
GHV35	GHANA	*N. m. monachus*	FEATHER	7.1393	0.4778	J. Deikumah
GVH24	GHANA	*N. m. monachus*	FEATHER	7.1464	0.4745	J. Deikumah
GHV9	GHANA	*N. m. monachus*	FEATHER	5.1321	-1.2924	J. Deikumah
HVGM01	GHANA	*N. m. monachus*	TOEPAD	51.0830	-13.4170	Naturalis biodiversity centre
HVA01	ANGOLA	*N. m. pileatus*	TOEPAD	-15.1083	13.9417	Naturalis biodiversity centre
HVU01	UGANDA	*N. m. pileatus*	TOEPAD	0.6250	33.4917	Naturalis biodiversity centre

Appendix 2.2: The genetic statistics of Hooded vulture (*Necrosyrtes monachus*) and *Necrosyrtes monachus monachus* and *Necrosyrtes monachus pileatus*. It indicates the number of alleles (A), the number of effective alleles (N_e), the null allele frequency (N_o), the observed heterozygosity (H_o), the unbiased expected heterozygosity (uHe), inbreeding coefficient (F), the polymorphic information content (PIC) and Shannon's information index (I) the deviation from the Hardy Weinberg Equilibrium (HWE) and The uncorrected and corrected null alleles (F_{ST})

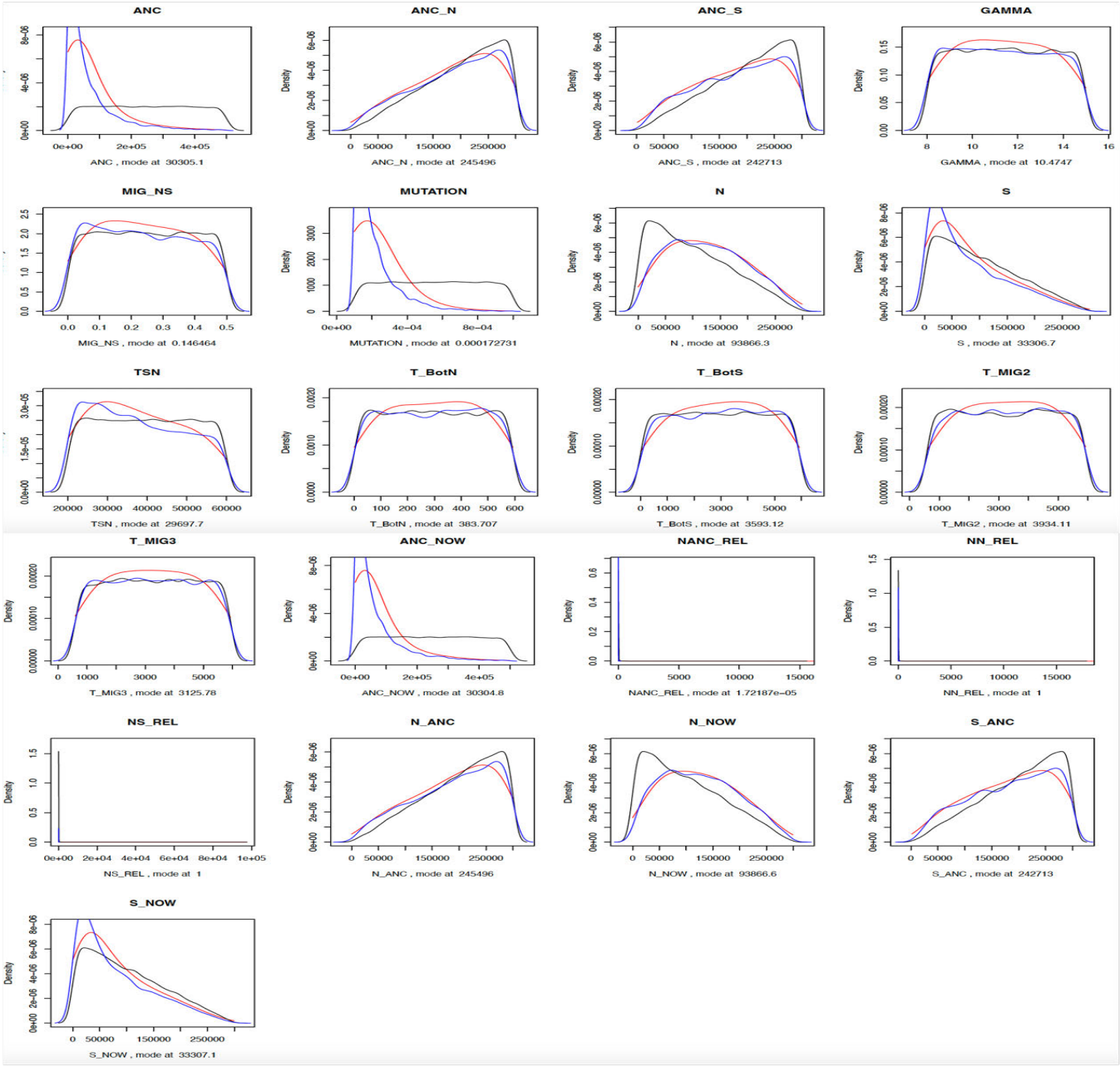
Species	**Locus**	**A**	**N_e**	**N_0**	**I**	**H_o**	**H_e**	**uHe**	**HWE**	**PIC**	**F**	**F_{ST}**
Necrosyrtes monachus	BV2	9.000	1.735	0.156	0.994	0.238	0.423	0.429	0.001	0.408	0.438	0.442
	BV 13	6.000	3.714	0.256	1.484	0.286	0.731	0.740	0.000	0.690	0.609	0.604
	Gf9C	12.000	7.200	0.046	2.164	0.810	0.861	0.871	0.000	0.846	0.060	0.051

	BV11	13.000	3.093	0.000	1.615	0.732	0.677	0.685	0.000	0.644	-0.081	0.095
	Gf8G	12.000	2.490	0.164	1.432	0.341	0.598	0.606	0.000	0.576	0.429	0.433
	BV17	7.000	1.947	0.249	1.094	0.156	0.486	0.494	0.000	0.467	0.679	0.687
	BV8	5.000	2.015	0.143	1.020	0.286	0.504	0.510	0.000	0.470	0.433	0.486
	BV 12	14.000	6.571	0.187	2.250	0.500	0.848	0.859	0.000	0.863	0.410	0.451
	Gf11A4	7.000	3.883	0.200	1.531	0.425	0.743	0.752	0.000	0.686	0.428	0.478
	BV9	7.000	1.679	0.252	0.823	0.071	0.404	0.409	0.000	0.367	0.823	0.825
	BV6	8.000	3.255	0.002	1.451	0.829	0.693	0.701	0.049	0.643	-0.197	0.172
	Gf3H3	9.000	4.009	0.044	1.607	0.643	0.751	0.760	0.028	0.710	0.144	0.181
	BV 20	8.000	2.832	0.000	1.404	0.550	0.647	0.655	0.000	0.615	0.150	0.167
	Mean	**9.000**	**3.417**	**0.131**	**1.452**	**0.451**	**0.643**	**0.652**	**0.000**	**0.612**	**0.333**	**0.390**
Necrosyrtes monachus monachus	BV2	4.000	1.995	0.254	0.927	0.150	0.499	0.512	0.000	0.472	0.699	0.708
	BV 13	4.000	2.930	0.307	1.205	0.150	0.659	0.676	0.000	0.614	0.772	0.776
	Gf9C	10.000	6.061	0.009	2.027	0.900	0.835	0.856	0.006	0.825	-0.078	0.036
	BV11	12.000	5.157	0.000	2.024	0.842	0.806	0.828	0.000	0.766	-0.045	0.030
	Gf8G	11.000	5.309	0.155	1.971	0.474	0.812	0.834	0.000	0.769	0.416	0.394
	BV17	4.000	2.404	0.339	1.087	0.063	0.584	0.603	0.000	0.537	0.893	0.899
	BV8	4.000	1.616	0.223	0.772	0.150	0.381	0.391	0.001	0.342	0.607	0.722
	BV 12	10.000	9.000	0.140	2.248	0.667	0.889	0.914	0.000	0.883	0.250	0.303
	Gf11A4	5.000	3.126	0.140	1.316	0.579	0.680	0.698	0.000	0.637	0.149	0.218
	BV9	7.000	2.694	0.287	1.240	0.150	0.629	0.645	0.000	0.572	0.761	0.764
	BV6	8.000	3.358	0.000	1.516	0.895	0.702	0.721	0.030	0.671	-0.274	0.225
	Gf3H3	7.000	3.556	0.006	1.486	0.650	0.719	0.737	0.172	0.679	0.096	0.149
	BV 20	7.000	3.760	0.000	1.556	0.632	0.734	0.754	0.005	0.689	0.140	0.183
	Mean	**7.154**	**3.920**	**0.143**	**1.490**	**0.485**	**0.687**	**0.705**	**0.000**	**0.651**	**0.337**	**0.416**
Necrosyrtes monachus pileatus	BV2	7.000	1.478	0.000	0.777	0.318	0.323	0.331	0.676	0.326	0.016	0.035
	BV 13	5.000	2.814	0.137	1.227	0.409	0.645	0.660	0.026	0.585	0.365	0.354
	Gf9C	8.000	4.768	0.035	1.750	0.727	0.790	0.809	0.458	0.756	0.080	0.055
	BV11	5.000	2.073	0.000	0.954	0.636	0.518	0.530	0.276	0.466	-0.230	0.231
	Gf8G	3.000	1.377	0.072	0.503	0.227	0.274	0.280	0.338	0.229	0.170	0.263
	BV17	4.000	1.484	0.990	0.672	0.250	0.326	0.337	0.075	0.308	0.234	0.263
	BV8	4.000	2.344	0.072	1.045	0.409	0.573	0.587	0.002	0.495	0.286	0.327

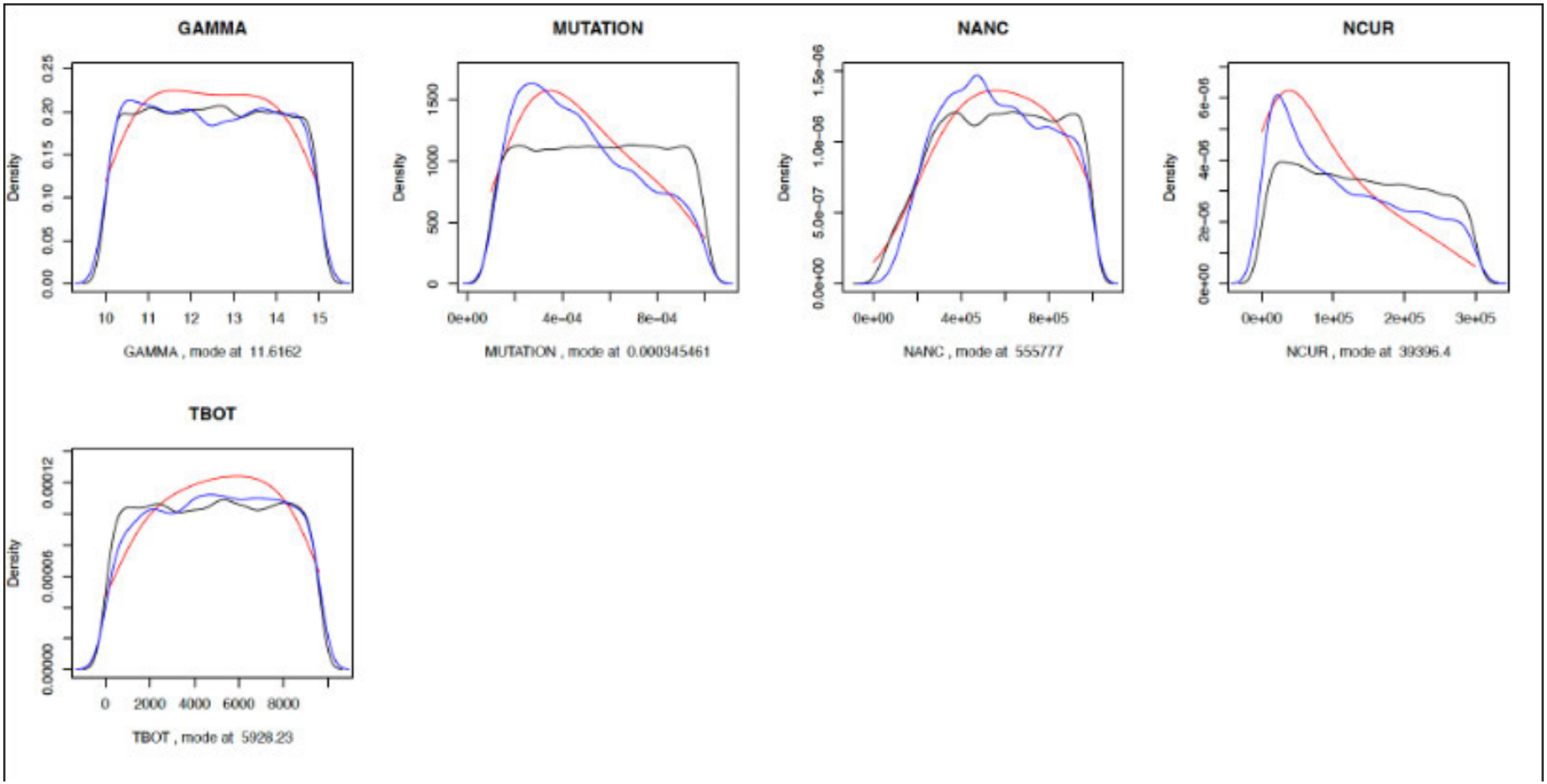
BV 12	9.000	3.103	0.204	1.547	0.364	0.678	0.693	0.00	0.647	0.463	0.525
Gf11A4	6.000	4.324	0.292	1.562	0.286	0.769	0.787	0.000	0.718	0.628	0.684
BV9	1.000	1.000	0.001	0.000	0.000	0.000	0.000	-	0.000	-	-
BV6	5.000	2.839	0.000	1.248	0.773	0.648	0.663	0.402	0.585	-0.193	0.164
Gf3H3	7.000	3.396	0.069	1.466	0.636	0.706	0.722	0.123	0.670	0.098	0.155
BV 20	4.000	2.018	0.000	0.929	0.476	0.505	0.517	0.154	0.428	0.056	0.066
Mean	**5.231**	**2.540**	**0.144**	**1.052**	**0.424**	**0.519**	**0.532**	**0.000**	**0.478**	**0.165**	**0.260**

Appendix 2.3: The comparison of membership coefficient (Q-values) of the two subspecies of Hooded vulture (*Necrosyrtes monachus monachus* and *Necrosyrtes monachus pileatus*) for the Admixture model used in STRUCTURE.

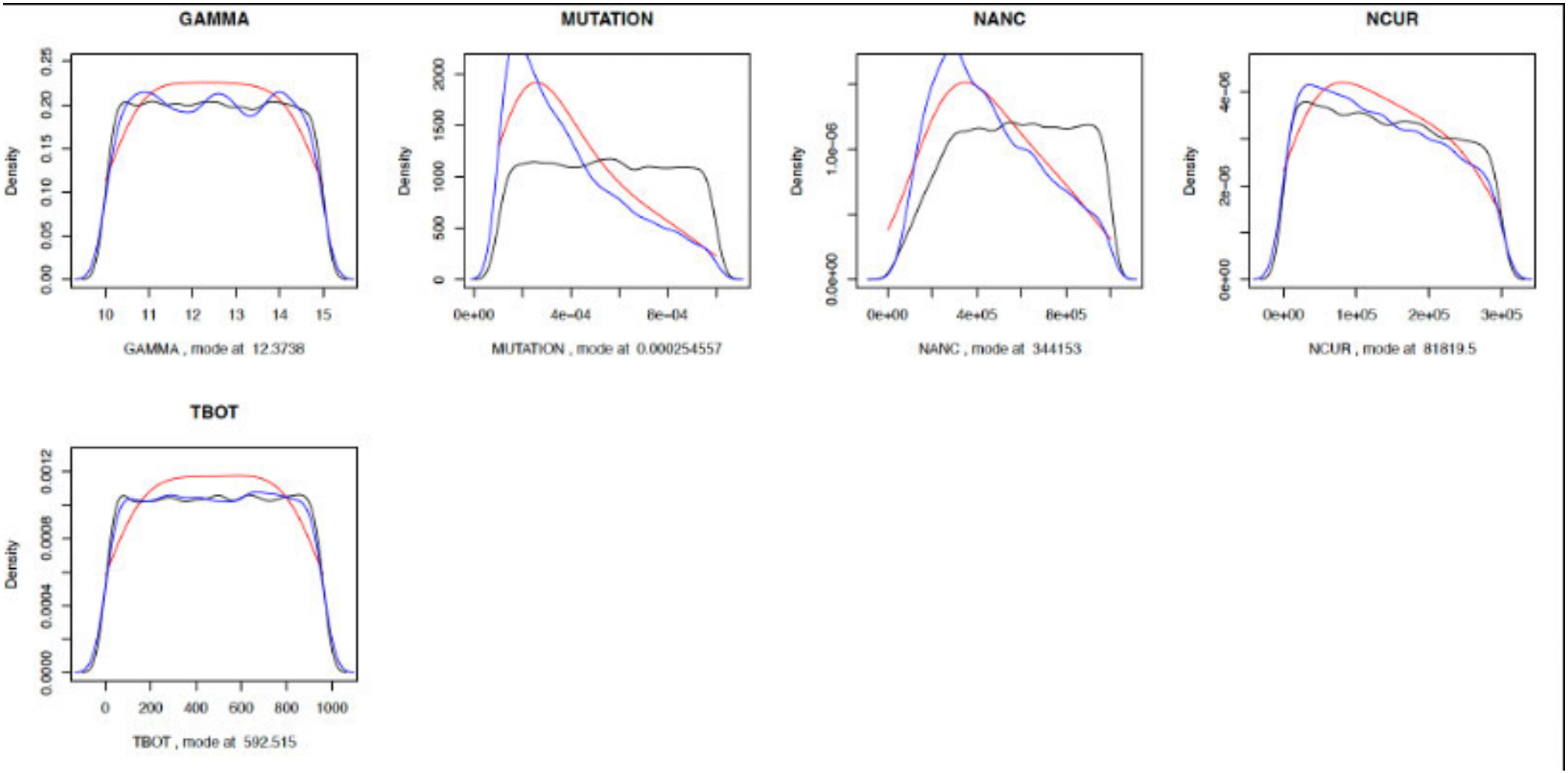
Sample origin	**Sample size**	**Admixture**
Necrosyrtes monachus monachus	20	0.206
Necrosyrtes monachus pileatus	22	0.200
Senegal	10	0.928
Ghana	10	0.7184
Ethiopia	9	0.800
South Africa	11	0.945
Angola	1	0.166
Uganda	1	0.287



Appendix 2.4 Posterior estimation for the Approximate Bayesian computation analyses done for the migration rates between the two subspecies of Hooded vultures (*Necrosyrtes monachus monachus* and *Necrosyrtes monachus pileatus*) where both subspecies experienced a bottleneck event and migration was bi-directional.



Appendix 2.5 Posterior estimation for the Approximate Bayesian Computation (ABC) analyses done to test for demographical changes the subspecies of Hooded vulture (*Necrosyrtes monachus pileatus*) where the subspecies was modelled to experience a ancient bottleneck.

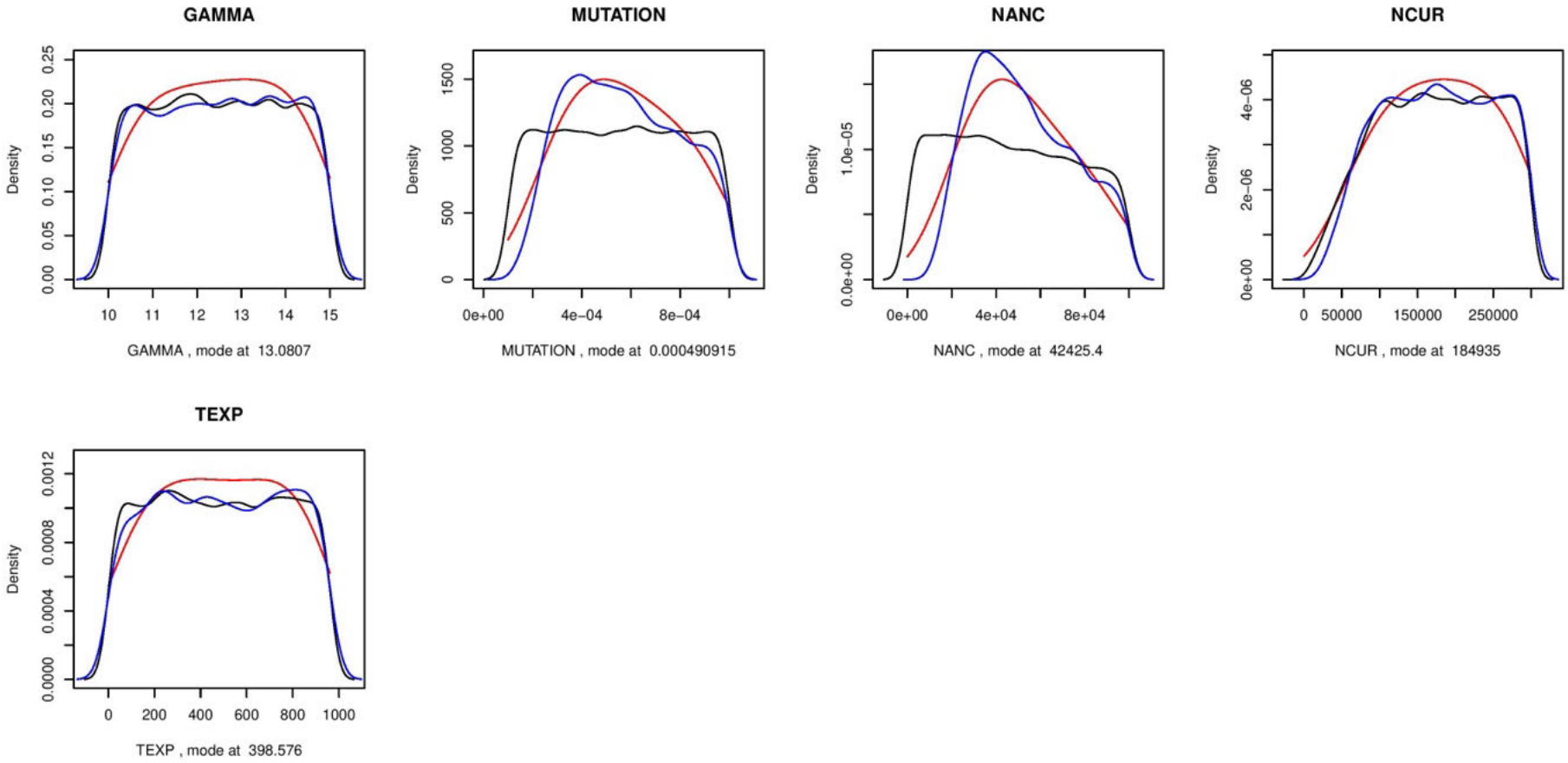


Appendix 2.6 Posterior estimation for the Approximate Bayesian Computation (ABC) analyses done to test for demographical changes the subspecies of Hooded vulture (*Necrosyrtes monachus monachus*) where the subspecies was modelled to experience a recent bottleneck.

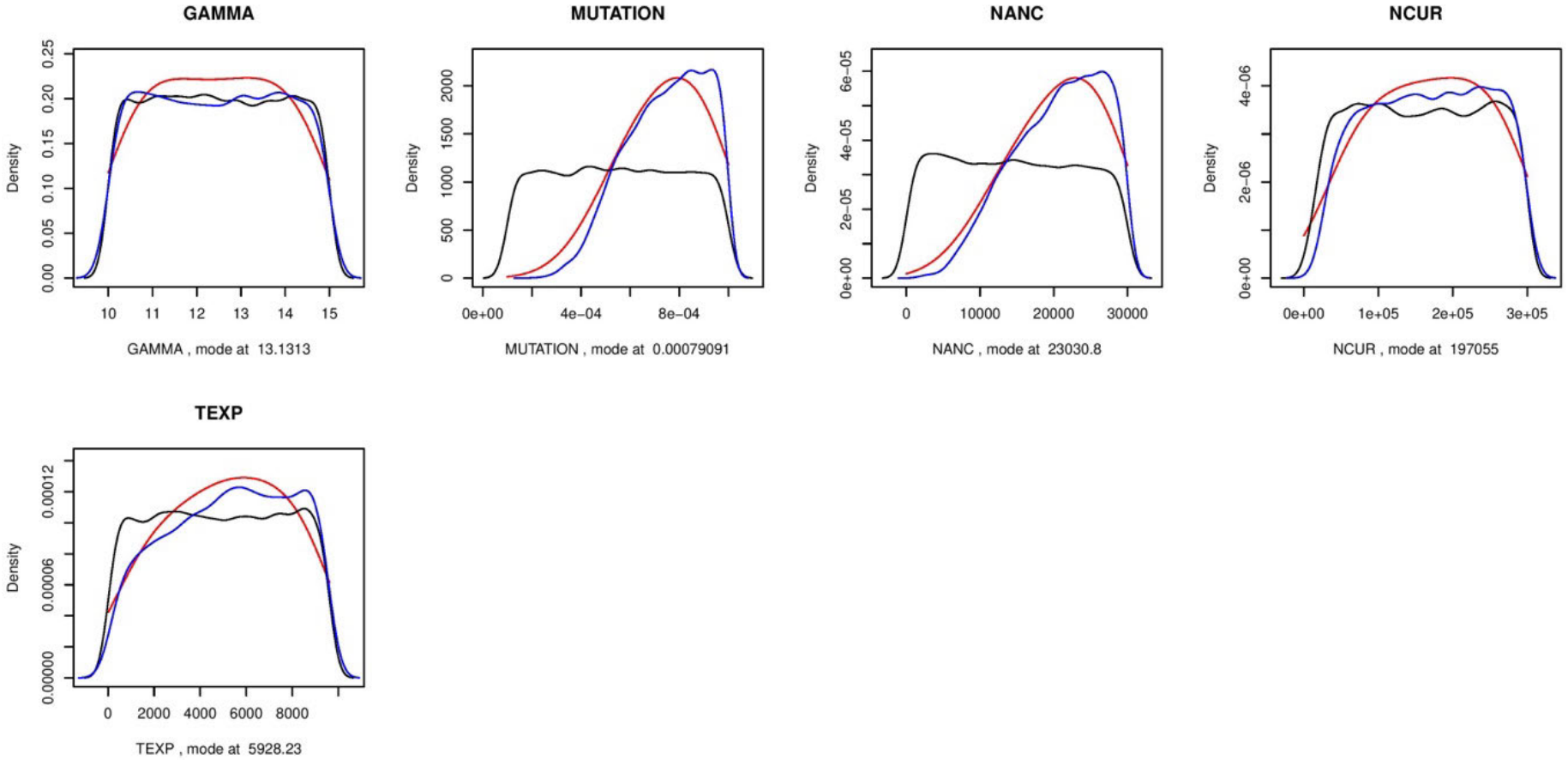
Appendix 3.1: Summary showing the statistics for each locus included in the analyses of Hooded vulture (*Necrosyrtes monachus*) from South Africa (n = 30) and Ghana (n = 30) genotyped in the current study. The number of alleles (A), number of effective alleles (Ne), null allele frequency (N_o), observed heterozygosity (H_o), unbiased expected heterozygosity (uHe), deviation from Hardy-Weinberg equilibrium (HWE) p-value, inbreeding coefficient (F) and polymorphic information content (PIC) of each locus are given.

Population	Locus	A	Ne	N_0	Ho	He	uHe	HWE	PIC	F
South Africa	BV2	4.000	2.871	0.121	0.433	0.652	0.663	0.000	0.000	0.335
	BV13	8.000	2.528	0.000	0.533	0.604	0.615	0.287	0.757	0.118
	Gf9C	14.000	7.155	0.096	0.708	0.860	0.879	0.001	0.802	0.177
	BV11	7.000	2.987	0.000	0.857	0.665	0.677	0.032	0.790	-0.289
	Gf8G	4.000	1.439	0.169	0.138	0.305	0.310	0.003	0.622	0.548
	BV17	6.000	1.250	0.090	0.143	0.200	0.204	0.114	0.577	0.287
	BV8	4.000	1.272	0.200	0.033	0.214	0.218	0.000	0.539	0.844
	BV12	12.000	6.143	0.000	0.833	0.837	0.851	0.237	0.729	0.005
	Gf11A4	9.000	5.128	0.273	0.310	0.805	0.819	0.000	0.894	0.614
	BV9	4.000	2.369	0.093	0.517	0.578	0.588	0.000	0.842	0.105
	BV6	13.000	7.563	0.008	0.833	0.868	0.882	0.000	0.759	0.040
	Gf3H3	6.000	3.930	0.000	0.862	0.746	0.759	0.368	0.861	-0.156
	BV20	7.000	2.572	0.153	0.345	0.611	0.622	0.000	0.724	0.436
	Gf3f3	5.000	4.083	0.216	0.379	0.755	0.768	0.000	0.788	0.498
	Mean	**7.357**	**3.664**	**0.101**	**0.495**	**0.621**	**0.632**	**-**	**0.692**	**0.254**
Ghana	BV2	5.000	2.635	0.346	0.067	0.621	0.631	0.000	0.571	0.893
	BV13	4.000	1.829	0.200	0.200	0.453	0.461	0.000	0.398	0.559
	Gf9C	13.000	10.714	0.058	0.800	0.907	0.922	0.006	0.899	0.118
	BV11	4.000	1.529	0.000	0.412	0.346	0.357	1.000	0.316	-0.190
	BV14	8.000	2.927	0.029	0.633	0.658	0.669	0.210	0.624	0.038
	Gf8G	4.000	1.403	0.000	0.300	0.287	0.292	0.320	0.262	-0.044

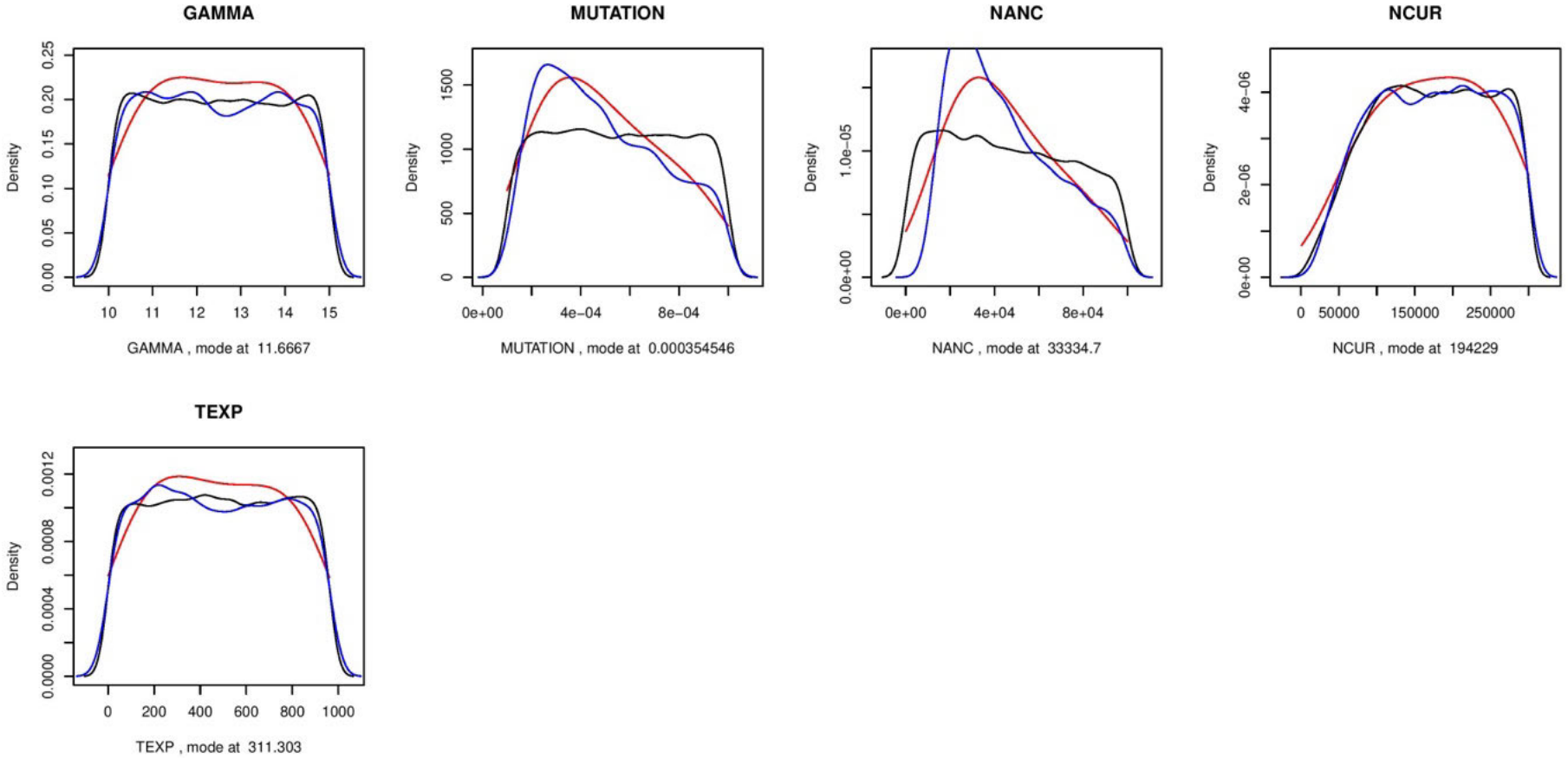
	BV17	2.000	1.080	0.132	0.000	0.074	0.075	0.020	0.071	1.000
	BV8	1.000	1.000	0.001	0.000	0.000	0.000	-	0.000	-
	BV9	4.000	1.151	0.106	0.069	0.131	0.134	0.035	0.128	0.475
	BV6	11.000	6.722	0.016	0.818	0.851	0.871	0.279	0.835	0.039
	Gf3H3	11.000	3.742	0.097	0.567	0.733	0.745	0.002	0.710	0.227
	BV5	4.000	1.328	0.095	0.182	0.247	0.253	0.045	0.236	0.264
	BV20	3.000	1.148	0.130	0.045	0.129	0.132	0.023	0.125	0.264
	Mean	5.692	2.862	0.093	0.315	0.418	0.426	-	0.398	0.335



Appendix 3.2: The best posterior estimations for the best-fitting Approximate Bayesian Computation (ABC) model (recent expansion) for the South African Hooded vulture population (*Necrosyrtes monachus*). These graphs indicate the ancestral population size, current effective population size, and generational time the event occurred.



Appendix 3.3: The best posterior estimations for the best-fitting Approximate Bayesian Computation (ABC) model (ancient expansion) for the South African Hooded vulture population (*Necrosyrtes monachus*). These graphs indicate the ancestral population size, current effective population size, and generational time the event occurred.



Appendix 3.4: The best posterior estimations for the best-fitting Approximate Bayesian Computation (ABC) model (recent expansion) for the Hooded vulture population (*Necrosyrtes monachus*) in Ghana. These graphs indicate the ancestral population size, current effective population size, and generational time the event occurred.