THE EFFECT OF GENOTYPE ON AVIAN MALARIA INFECTIONS IN THE AMUR FALCON (*Falco amurensis*)

by

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PREFACE

The research contained in this thesis was completed by the candidate while based in the Discipline of Genetics, School of Life Sciences of the College of Agriculture, Engineering and Science, University of KwaZulu-Natal, Campus Pietermaritzburg, South Africa. The research was financially supported by National Research Foundation.

The contents of this work have not been submitted in any form to another university and, except where the work of others is acknowledged in the text, the results reported are due to investigations by the candidate.

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Signed: Prof S. Willows-Munro

Date: 9 February 2022

DECLARATION: PLAGIARISM

I, Rachel Caitlin Stoffberg, declare that:

(i) the research reported in this thesis, except where otherwise indicated or acknowledged, is my original work;

(ii) this thesis has not been submitted in full or in part for any degree or examination to any other university;

(iii) 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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a) their words have been re-written but the general information attributed to them has been referenced;

b) where their exact words have been used, their writing has been placed inside quotation marks, and referenced;

(v) where I have used material for which publications followed, I have indicated in detail my role in the work;

(vi) this thesis is primarily a collection of material, prepared by myself, published as journal articles or presented as a poster and oral presentations at conferences. In some cases, additional material has been included;

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ABSTRACT

Avian malaria is caused by haemosporidian parasites (*Plasmodium*, *Haemoproteus* and *Leucocytozoon*) that are transmitted by dipteran vectors. Passerines have been the focus of avian malaria research however raptors are generally keystone species in ecosystems making them important hosts to investigate. The Amur falcon (*Falco amurensis*) is a small raptor with the longest migration recorded in any raptor species. This host is particularly interesting to investigate as although it is a raptor it belongs to the order Falconiforms which ultimately is more closely related to parrots and passerines compared to other raptors. The falcons congregate in large flocks during migration which may impact the infection rate of the parasites. The Amur falcon has had a depletion in numbers due to mass harvesting in 2012 as well as two hailstorms that killed approximately 1000 falcons in Kwa-Zulu Natal, South Africa, making it a novel host to test for bottleneck events as well as genetic diversity and population structure. The main aim of this thesis was to determine the significance of age, sex and individual heterozygosity on avian malaria infections in the Amur falcon.

The results of this study indicated that the Amur falcon had a high rate of haemosporidian parasite infection, particularly *Haemoproteus*. Phylogenetic analyses indicated that *Haemoproteus* was host specific while *Leucocytozoon* was found to be more generalist, infecting many different species of birds. The Amur falcon population had high genetic diversity and low levels of inbreeding indicating a healthy population. There was a lack of population structure. Generalized linear models were used to test whether sex (male or female), age (juvenile or adult) and individual heterozygosity were drivers of avian malaria infection in the Amur falcon. No significant associations were found except when the different lineages of *Haemoproteus* were considered independently.

The data and results presented in this thesis provide a baseline for future studies on the Amur falcon, and also contributes towards a growing body of work examining haemosporidian parasite infections in migratory birds.

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CHAPTER 1: LITERATURE REVIEW

1.1 Abstract

Avian malaria is caused by three genera of haemosporidian parasites (*Plasmodium, Haemoproteus,* and *Leucocytozoon*). Haemosporidian parasites are extremely diverse, and infections caused by these parasites have been shown to be harmful to the host's health. However, little research has been conducted on parasites infecting many species of birds, with most research focused on passerines with limited data available on parasites infecting raptors. Raptor species are at the top of the food chain and are often keystone species within ecosystems making them important hosts to investigate. Parasites infecting migratory species are also an important focus of this study as migratory hosts can introduce parasites to novel environments that can lead to changes in local host-parasite dynamics. The prevalence of all three genera of haemosporidian parasites in the Amur falcon (*Falco amurensis*) are investigated in this study. The Amur falcon is a small intercontinental migratory species that breeds in Eurasia but migrates to Southern Africa during winter. This study aims to examine the genetic diversity of avian malaria parasites infecting the Amur falcon and also understand how sex, age, and individual heterozygosity could be potential factors impacting haemosporidian infection status in this species. In the second chapter of this thesis, PCR-based methods were used to detect the parasite mitochondrial cytochrome *b* (cyt *b*) gene which was used to delimit the three genera of avian malaria parasites. Other Southern African raptor species were also screened to determine host-parasite specificity and determine what impact the migratory behaviour of the falcons was having on local raptor parasite populations. Evolutionary theory highlights that genetic diversity within a population is essential for the population's resilience in the face of emerging pathogens. In the third chapter of this thesis, eight microsatellite loci were used to determine the genetic diversity present in the KwaZulu-Natal population of the Amur falcon and were also used to test for the presence of population structure in species. In the fourth chapter, generalized linear models were used to determine if sex, age, and individual heterozygosity are linked to haemosporidian infection in this migratory raptor. This study adds to a growing body of work examining host-parasite interactions in birds and makes an important contribution towards wildlife parasitology.

1.2 Introduction

Avian haemosporidians blood parasites have been described from many different bird species across the globe (Ciloglu et al, 2016). Vectors such as biting midges, louse flies, black flies, and mosquitoes are responsible for transmitting haemosporidian parasites (Valkiunas, 2004). Avian haemosporidian parasites include members of the Plasmodiidae, Haemoproteidae, and Leucocytozoidae families (Ciloglu et al, 2016). Many undescribed species belong to these three parasite families, there are more than 2000 unique genetic haplotypes of avian haemosporidians species identified and recorded that still need to be formally described (Bensch et al, 2009; Clark et al, 2014; Valkiunas, 2004; Valkiūnas et al, 2005). Species discovery has been accelerated due to molecular technology (Clark et al, 2014). These blood parasites are ideal organisms for the study of wildlife parasitology due to the diversity and abundance of the parasites (Bensch et al, 2013; Valkiunas, 2004). Avian species provide important services such as pollination, seed dispersal and the maintenance of sustainable population levels of prey and predator species (Anderson et al, 2011). As a result, many bird species and in particular raptors are keystone species within their ecosystems (Mills et al, 1993). Falconiformes are an order of falcons that are more closely related to Parrots (Psittaciformes) and Passerines (Passeriforms) despite being classified as a raptor (Hackett et al, 2008; Wink et al, 1998). This makes them an interesting study species to compare to other raptors in order to determine if vector borne parasites infect both Accipitriformes, Strigiformes and Falconiformes.

Studies have shown that avian haemosporidian infections can be harmful to host species (Remple, 2004) by causing conditions such as anaemia and tissue damage which can result in reduced energy(Dawson & Bortolotti, 2000; Remple, 2004; Vogel, 2015). An increase in avian haemosporidian infections could have a cascade effect on ecosystems due to potentially high mortality rates in bird populations (LaPointe et al, 2012). Haemosporidian parasites can drive selection pressure within host species (Outlaw & Ricklefs, 2010) which in turn can act as selection pressure driving genetic diversity as a species needs to adapt to survive (Nei, 2005). Genetic diversity is an important factor in population health (Reed & Frankham, 2003). Populations with high genetic diversity are less susceptible to emerging pathogens (Lande, 1988; Primack, 2006). Pathogens such as the avian haemosporidian parasites may be a key driver of genetic diversity in hosts (Spurgin & Richardson, 2010). There are several ways to measure Genetic diversity. Microsatellite markers are one of the most popular methods used to genotype individuals allowing for the genetic diversity of a population to be measured (Chistiakov et al, 2006). Microsatellite loci are usually found within non-coding regions of the

genome or within sections of the genome which are not undergoing selection. As such microsatellite loci are usually (but not always) considered selectively neutral (Brohede & Ellegren, 1999; Schlötterer & Wiehe, 1999). Microsatellite data can be used to determine population structure which may hold valuable information on evolutionary processes occurring below the species level. Variation at microsatellite loci can also be used as a proxy for individual heterogeneity.

Demographic factors such as sex and age of the host may also affect prevalence of the haemosporidian parasites and susceptibility of individual hosts to disease (Hammers et al, 2016; Santiago-Alarcon et al, 2019; Slowinski et al, 2021). Adult birds have been the main focus of many studies resulting in a lack of information on how infections affect younger birds. Juvenile birds are still developing their immune system so are often targets of blood parasites (Atkinson et al, 2001) as such juveniles are expected to have higher parasite prevalence and more health concerns (Granthon & Williams, 2017). This is expected as a trade-off is made between growth and immune response making younger birds vulnerable to infection (Soler et al, 2003). Nestlings may be particularly vulnerable as they are confined to the nest making them more available to vectors. They also rely on parent for nutrients (Merino, 2010). Nutrition is vital in order to build immunity and have a chance of survival when exposed to infections (Hoi-Leitner et al, 2001; Lochmiller et al, 1993; Merino, 2010). Due to these reasons, it is expected that younger birds will have a higher infection rate compared to adults.

Gender has been considered as an important factor in haemosporidian infection due to the role of hormones in immune response. The immunocompetence hypothesis predicts that testosterone will supress the immune response while controlling and enhancing sexual signals in males. This may result in males being more susceptible to haemosporidian parasites (Folstad & Karter, 1992). Studies have shown conflicting results, with some studies showing that age and sex are significant for infection status (Isaksson et al, 2013), while other studies found no significant correlation (Ágh et al, 2019; Fecchio et al, 2015; Marzal et al, 2008).

1.3 Avian malaria

There are two phases in avian malaria infection which vary greatly depending on the parasites present and the host species infected (Santiago-Alarcon et al, 2012). In the acute phase, There is a high density of the parasite in the host's blood which may result in some birds succumbing to the infection (Santiago-Alarcon et al, 2012). The birds that succumb to infection never reach the chronic phase which hinders research on the effect of the parasite on

host fitness (Asghar et al, 2011). Chronic infection is often exhibited during the breeding season (Knowles et al, 2010) because during this time birds suppress their immune system in favour of increased reproductive effort. However, some infected birds may show the same fitness as non-infected birds (Manwell, 1934). The lack of physical symptoms will contribute significantly to the spread of the disease (Asghar et al, 2011).

Other factors such as host immunocompetence, host density, proximity to water, temperature, and climate may also play an important role in the transmission rate of avian malaria among potential hosts (LaPointe et al, 2012). Birds that nest communally are predicted to increase the spread of the parasites (Santiago-Alarcon et al, 2012). Climate may also impact transmission as some vectors are confined to or are more abundant in warmer climates (Lachish et al, 2011). As such, global warming is expected to increase the transmission of avian malaria (LaPointe et al, 2012). For example, the increase in temperature linked to global warming has been linked to the spread of avian malaria in New Zealand (LaPointe et al, 2012). Another contributing factor is some birds not displaying physical symptoms in the chronic phase during the breeding season which could lead to increased infection rates (Ellis et al, 2014).

1.3.1 Three genera of haemosporidian parasites

There are three main genera of haemosporidian parasites (*Plasmodium*, *Haemoproteus*, and *Leucocytozoon*) that cause avian malaria in birds. At least 50 species have been described that cause avian malaria (Santiago-Alarcon et al, 2012). Parasite species differ in pathogenicity, host range, geographical distribution and vectors (Santiago-Alarcon et al, 2012).

The complete life cycle of avian malaria parasites requires both a host and a vector (Ricklefs et al, 2004). The parasites reproduce both asexually and sexually in the vector and sexually in the vector (Ferraguti et al, 2013). The relationship between the vector and the parasite is unclear and the life cycles of the parasites are not fully understood (Ferraguti et al, 2013). There is abundant research supporting the occurrence of host switching in these parasites (Ricklefs & Fallon, 2002; Ricklefs et al, 2004; Szymanski & Lovette, 2005). This indicates that parasite lineages may be passed on to bird hosts of different species resulting in the spread of the avian malaria parasites (Bensch et al, 2000; Križanauskienė et al, 2006; Ricklefs & Fallon, 2002; Waldenström et al, 2002). It is for this reason that studying the avian malaria infection rate in migratory birds is so important. These hosts may be transporting avian malaria parasites around the globe.

An important consideration in studying avian malaria transmission is understanding how common each genus of the parasite is in different bird species. In research conducted

primarily on passerines, Plasmodium is the most prevalent (Bensch et al., 2000) due to its broad host range (Valkiūnas & Iezhova, 2018), followed by *Haemoproteus* (Bensch et al., 2000). *Leucocytozoon* was the least prevalent (Bensch et al., 2000).The parasite genera are transmitted by many species of mosquitoes including species belonging to the genera *Anopheles, Mansonia, Culex, Culisetta, Aedes, Psorophora,* and *Coquilettidia* (Valkiūnas & Iezhova, 2018). A high mortality rate has been reported in birds infected with *Plasmodium* (Van Riper et al, 1986), but the impact of *Plasmodium* on pathogenicity and the host's health is not fully understood (Van Riper III et al, 1986). The transmission of *Plasmodium* parasites can occur in polar regions indicating that low temperatures do not hinder transmission (Valkiūnas & Iezhova, 2018). *Plasmodium* can be spread in both warm and cold regions which is concerning. Asexual and sexual processes are involved in the life cycle of the parasite (Valkiunas, 2004). The high prevalence of *Plasmodium* in passerine species may be explained due to the parasites asexually reproduction in the peripheral blood and the secondary monogony phase (Valkiunas, 2004). Although less well-studied in raptors, given the life history traits of this genus of parasites it is predicted that members of this genus will also show a high prevalence in raptors.

1.3.2 Methods of identifying haemosporidian parasites

Microscopy is the traditional method of identifying haemosporidian parasites. Although it can identify parasites in blood smears and is cost effective, it also requires taxonomic training and mixed parasite lineages can be difficult to identify (Waldenström et al, 2004). Highly sensitive and accurate molecular methods that amplify genes specific to the parasite may assist in morphological detection (Feldman et al, 1995; Ribeiro et al, 2005). Unique lineages may be identified by phylogenetic analysis of the parasite sequences which may be undescribed species (Waldenström et al, 2004). PCR methods generally utilize either the regions of the ribosomal DNA (Fallon et al, 2003; Feldman et al, 1995; Richard et al, 2002) or a portion of the cytochrome *b* gene (Ciloglu et al, 2016). Both of these markers are on the mitochondrial genome. Cytochrome *b* analyses have a higher detection rate compared to the ribosomal DNA markers (Waldenström et al, 2004).

1.4 Amur falcon (*Falco amurensis*)

Migratory species are an important consideration in the study of haemosporidian infections (Levin et al, 2013; Waldenström et al, 2002). Migratory species are particularly important to study as they may carry these parasites to many different continents and may facilitate disease outbreaks (Bildstein, 2006). Raptors are an interesting case study for studying

haemosporidian infections in migratory species as only 62% of them migrate (Bildstein, 2006). Birds that migrate travel through many environments and climates (Bildstein, 2006) and are exposed to a wide variety of different vectors. The Amur falcon (*Falco amurensis*; Naoroji & Schmitt, 2007), can survive in many different environments due to their broad dietary base making them opportunistic in terms of where they are distributed (Symes & Woodborne, 2010). It is thought that the Amur falcon spends winter in southern Africa and breeds in Asia however their migration patterns are not well documented (Ganpule, 2011). A recent study revealed that the Amur falcon takes the longest migration of any raptor species – birds with transmitters travelled over 5 912 kilometres non-stop over five days from Somalia to India (Meyburg et al, 2017). Mass harvesting of this species occurs in India which has resulted in a depletion of numbers (Bouwman et al, 2012). When migrating the Amur falcons congregate in large flocks that may impact the spread of the parasite (Ali & Ripley, 1980).

1.5 Genetic diversity driven by pathogens

Genetic diversity within a population has been shown to be linked to population health and fitness (Westemeier et al, 1998). Genetic diversity is the heritable variation within a population (Humphries et al, 1995). This variation is measured by values such as the number of alleles, heterozygosity, and heritability of traits (Frankham et al, 2002). It is believed that one of the mechanisms to maintaining genetic diversity in wild populations is by pathogen mediated selection (Spurgin & Richardson, 2010). This type of selection involves a pathogen causing the selection of certain alleles or genotypes that moderate pathogen virulence (Ford, 2002; Spurgin & Richardson, 2010). Individual genetic diversity or heterozygosity may also impact disease resistance. It is expected that the more heterozygous an individual's genome, the less susceptible to diseases (Lively, 2010). Again studies have had contradictory results, some studies have shown that increased individual heterozygosity is a benefit in staving off infection (Albeshr, 2016; Townsend et al, 2018) while others showed no significant link between individual heterozygosity and parasite infection status (Ferrer et al, 2014; Kubacka et al, 2020; Vallender et al, 2012) .

This MSc study will examine genetic diversity of both host and parasite. Genetic diversity of the host (Amur falcon) will be measured using a suite of microsatellite loci. Microsatellites are co-dominant markers that have a high level of polymorphism making them useful in forensics, population genetics, genetic mapping, and evolutionary studies (Chistiakov et al, 2006). They are relatively small in size (Jarne & Lagoda, 1996). These short tandem repeats (STR) span the genome and are repeated between 5-50 times. Microsatellite loci are

genome wide and generally located in non-coding regions such as intergenic spaces, in introns and untranslated regions (UTR), but they can occur in coding exonic sequences (Selkoe & Toonen, 2006). Microsatellite markers have been used on other species belonging to the *Falconidae* family (Magonyi et al, 2019a; Nesje et al, 2000b), however no study has focused on microsatellite analyses in Amur falcon populations, which makes this study novel.

1.6. Effect of migration on genetic diversity and population structure

There are two theories explaining how migratory behaviour can affect genetic diversity (Berthold, 1991; Willoughby et al, 2017). The first is that migration will result in high levels of genetic diversity due to the broad dispersal of the individuals and the increase in gene flow across populations (Lees & Gilroy, 2014; Zink et al, 2006), the other theory is that migration will homogenise genetic diversity (Alcala et al, 2013; Tollington et al, 2013; Wade & McCauley, 1988) Limited migration allows for differentiation between populations which results in increased genetic diversity while strong migration may homogenise genetic diversity (Alcala et al, 2013). However, it has been proposed that long distance migrants may have increased genetic diversity due to balancing selection on genes controlling migratory behaviour (Fitzpatrick, 1994). Migrants may also be exposed to different environments and more diverse parasites which could also result in increased genetic diversity (Møller et al, 2011; Møller & Erritzøe, 1998). Some migrants may overshoot their targeted destination and have to breed in smaller fragmented populations which may result in viable allopatric populations that could result in increased genetic differentiation and population structure (Lees & Gilroy, 2014). In general raptors have high fidelity to breeding regions (Rosenfield & Bielefeldt, 1996; Steenhof et al, 2005) which may result in strong population structure. In contrast, studies on other long-distance migrants have shown a lack of population structure (Kvistad et al, 2015; Ogden et al, 2015). It is unclear if seasonal migration promotes genetic diversity or reduces it as there are conflicting theories in the literature (Battey, 2018). This makes the Amur falcon a novel host to study genetic diversity and population structure as it has the longest migration recorded in raptors.

1.7 Main aims of this MSc study

The main aim of this study is to examine how biological factors such as sex, age and individual heterogeneity may influence avian malaria infection in a large sample of the Amur falcons. Each chapter is written as a manuscript for publication and as such there may be some repetition, although every attempt has been made to reduce this.

In the second chapter, I use a PCR-based method to determine avian malaria prevalence (*Plasmodium*, *Haemoproteus* and *Leucocytozoon*) in a large sample of Amur falcons. In addition to the Amur falcon, a selection of other southern African raptors will be screened, and the parasite data combined with that of previous studies to determine if the parasite lineages affecting the Amur falcons are species-specific or if extensive host-shifts have occurred. Phylogeny and haplotype network analyses will be used to determine the genetic diversity of parasites infecting the Amur falcons. In the third chapter eight microsatellite loci were used to measure genetic diversity and population structure in the KwaZulu-Natal Amur falcon population. The genetic diversity of the Amur falcons will be compared to closely related species as well as non-migratory species in order to determine the effect of migration gene flow on genetic diversity and population structure. Using genetic data, I will also test for signatures of recent population bottlenecks and determine the effective population size. In the fourth chapter I use general linearized models to determine the effect of sex, age and individual heterozygosity on haemosporidian infection status in the Amur falcons. Five heterozygosity indices will be calculated from the microsatellite data collected in Chapter three as measures of individual heterozygosity.

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CHAPTER 2: Prevalence, biodiversity, and specialisation of haemosporidian parasites infecting the Amur falcon (*Falco amurensis*)

Abstract

The Amur falcon is an intercontinental migratory species that makes yearly migrations between Asia and Southern Africa. The broad distribution of the species, broad habitat niche, and communal roosting make it an ideal model species to study host-parasite interactions. Three genera of haemosporidians malaria parasites (*Plasmodium*, *Haemoproteus*, and *Leucocytozoon*) infect birds. One hundred and seventy-eight Amur falcons killed in KwaZulu-Natal, South Africa were screened for the three haemosporidian parasite genera using a nested PCR method. Other raptor samples collected from South Africa were also included in analyses to determine if any lineages of parasites are locally endemic. Phylogenetic and network analyses were performed to determine the diversity of parasites. Only one Amur falcon was infected with *Plasmodium*. Sixty Amur falcons were found to be infected by *Haemoproteus.* Phylogenetic analyses of these parasite sequences recovered two distinct lineages. The results showed that these two lineages of *Haemoproteus* were host specific. One lineage infected only Amur falcons while the second lineage infected Amur falcons, as well as lesser kestrels which both belong to the same genus. *Leucocytozoon* infections were found in five Amur falcons and phylogenetic analyses of parasite sequences indicated that this parasite genus was not host-specific as the same parasite lineage was also recovered from four other bird species (*Falco sparverius*, *Circus aeruginosus*, *Tyto alba* and *Aegypius monachus*). This study provides important baseline data for future studies of parasites in this migratory species.

2.1 Introduction

Haemosporidian parasites have been reported across the globe in many different avian hosts (Ciloglu et al, 2016). Avian malaria is one of the most significant parasitic diseases in birds (Atkinson et al, 2009). The parasites causing malaria in birds belong to three families: Haemoproteidae, Plasmodiidae, and Leucocytozoidae (Ciloglu et al, 2016). These parasites are primarily transmitted by insects belonging to the order Diptera such as mosquitoes and biting midges (Valkiunas, 2004). Many species of avian malaria remain undescribed making it a key field to research

Malaria infections can pose a significant risk to bird populations resulting in harmful effects. These effects can vary and include tissue damage (Desser & Bennett, 1993), as well as

anemia. Some studies showed a lack of available resources in infected birds resulting in a poorer condition compared to infected birds (Dawson & Bortolotti, 2000) ;;Elevated mortality rates in bird populations due to haemosporidian infections may have a cascade effect on ecosystems (LaPointe et al, 2012). The diversity well as abundance of these vector borne parasites make them a significant topic for research. The impacts of these infections on both passerines as well as raptors have a significant impact on the ecosystem and could result in cumulative effects. .

The prevalence of parasite species infecting birds depends on factors such as host specificity, host range, presence of insect vectors, and pathogenicity (Santiago-Alarcon et al, 2012). In research dominated by studies involving passerines, *Leucocytozoon* was generally found at the lowest frequency, while *Plasmodium* is considered the most prevalent parasite infection followed by *Haemoproteus* (Bensch et al, 2000; Jia et al, 2018). It has been suggested that *Plasmodium* is particularly common due to its wide host range with the same *Plasmodium* species infecting many different bird species (Jia et al, 2018)**.** There is however a growing body of evidence suggesting that the relative frequency of the three genera of malaria in host species is not consistent across the avian phylogeny. For example, in a recent study of 13 raptor species from three families (Accipitridae, Falconidae, and Strigidae) in Iran, *Plasmodium* was not found to be the most prevalent, instead *Haemoproteus* and then *Leucocytozoon* were found to be dominant (Nourani et al, 2020). A study including 146 cinereous vultures (*Aegypius monachus*), 128 griffon vultures (*Gyps fulvus*), and 114 Egyptian vultures (*Neophron percnopterus*) found only *Leucocytozoon* present (Chakarov & Blanco, 2021). In another example, in Eleonora's falcon *(Falco eleonorae) Haemoproteus* was found to be the most prevalent malaria parasite infection followed by *Plasmodium*, while *Leucocytozoon* was found to be the least prevalent (Gutiérrez-López et al, 2015). Understanding the prevalence of avian malaria has important implications as *Plasmodium* infection is linked to higher mortality rates in birds (Van Riper et al, 1986).

Host specificity is also poorly understood among most species of avian malaria. Host switching has been linked to changes in virulence and so understanding which parasitic species are generalist or specialist may be an important consideration when identifying future emerging pathogens (Ricklefs & Fallon, 2002). Parasites with low host specificity (able to infect a large diversity of host species) are of special interest in disease ecology, as they are the most likely to be able to overcome ecological or evolutionary constraints to infect new hosts. A further complication is that for many parasite species host specificity is not a fixed trait but can vary in response to both evolutionary and environmental forces (Clark et al, 2018; Fecchio et al,

2019; Kamiya et al, 2014; Ricklefs et al, 2014). For example, the risk of *Plasmodium* infection in birds is expected to increase with increasing global temperatures (Garamszegi, 2011). Similarly, ecological factors such as differences in host dispersal capability can also drive variation in host specificity (Pérez-Tris & Bensch 2005; Ellis et al. 2015; Clark et al. 2017; Fecchio et al. 2018). In this regard migrant hosts may play a key role in the transport of parasites over large geographic distances and the transfer of parasites from other regions may impact the local prevalence and ecology of parasites (De Angeli Dutra et al, 2021). Nonetheless in general parasite-host specificity is phylogenetically linked, with parasite species infecting phylogenetically clustered subsets of available avian hosts (Beadell et al, 2004; Cooper et al, 2012; Poulin & Mouillot, 2005). The three genera of parasites differ in host specificity. *Plasmodium* species are considered more generalist while *Haemoproteus* species are often more host-specific (Beadell et al, 2004; Rhim et al, 2018). If this hypothesis is true, then the phylogenies of *Plasmodium* and *Haemoproteus* are expected to be quite different with the genetic structure of *Haemoproteus* more strongly correlated with that of their hosts.

Co-infection (concurrent infection with multiple genera of parasites) is another factor that needs to be considered. Infection can alter host susceptibility and interactions among parasites may be synergistic or antagonistic. Several studies have reported increased virulence in experimentally co-infected individuals (De Roode et al, 2005; Van Rooyen et al, 2013) but this effect may be host-species specific (Palinauskas et al 2011). In contrast, Marzal et al. (2008) showed that although co-infection resulted in increased mortality, it also increased reproductive success in house martins (*Delichon urbicum*), probably due to increased investment in reproduction. Davidar and Morton (2006) reported that a co-infection of *Haemoproteus* and filarial nematodes in purple martins (*Progne subis*) frequently resulted in the death of the host despite single infections of these parasites being relatively harmless. The differences observed may be a result of two levels of interaction: within-host competition between different parasite lineages and host immune defence. The three genera of avian malaria (*Plasmodium*, *Haemoproteus*, and *Leucocytozoon*) offers a unique model system for studying within-host interactions and evolution.

This study has two main aims. First, to test for the presence of *Plasmodium*, *Haemoproteus*, and *Leucocytozoon* in a large sample of Amur falcon (*Falco amurensis*) and the prevalence of these three genera. The Amur falcon is an intercontinental migratory raptor. Breeding in Asia and over-wintering in Southern Africa, the species undertakes one of the longest migrations of any raptor each year (Ganpule, 2011). The broad distribution of the species (breeding range far greater than ~ 4,000,000 km^2 in Northern Hemisphere and ~

500,000 km^2 in Southern Africa;), broad habitat niche (Symes and Woodborne, 2010), and use of communal roosting sites often containing thousands of individuals (Benson, 1951; Cade & Digby, 1982; Tarboton & Allan, 1984) make it a good model species to study host-parasite interactions. The Amur falcon is also a key host to determine if these vector-borne parasites infect the order, Faloniformes as well as other raptors belonging to the orders Accipitriformes and Strigiformes. Second, I aim to test for host specificity and determine the impact of migration connectivity on local raptor populations, by combining parasite sequences from Amur falcon with parasite sequences from other South African raptor species in phylogenetic analyses. Parasite sequences from raptors were also downloaded from GenBank. By including parasite sequences from other raptor species around the world I also hope to investigate whether any parasite lineages are endemic to South Africa. The phylogenies presented will also be used to examine the genetic diversity of parasites infecting the Amur falcon.

2.2 Materials and methods

2.2.1 Sampling

The transmission of haemosporidian parasites is affected by many abiotic and biotic factors and can vary in both space and time (Lachish et al, 2011; Loiseau et al, 2010). Biotic factors such as age and sex have also been shown to impact infection rates (Deviche et al, 2005; Hudson & Dobson, 1997; Van Oers et al, 2010). The 178 Amur falcon samples used in the study were all collected from two mass mortality events (Supplementary Table 2.1). This sample thus represents a snapshot of infections in the population, with the sampling not biased by temporal shifts in infection. On the 10 and 21 March 2019, hundreds of Amur falcons were killed in two hailstorms in the KwaZulu-Natal midlands (Mooi River and Newcastle). The birds were stored, and liver samples were taken at the Durban Natural Science Museum for genetic analyses. In addition to the Amur falcon samples, other raptor samples collected in South Africa were also screened (Supplementary Table 2.2). These samples were already available in the lab from past studies. These included: Cape vulture (*Gyps africanus,* n = 73), crowned eagle (*Stephanoaetus coronatus*, n = 3), white-backed vulture (*Gyps africanus*, n = 111), jackal buzzard (*Buteo rufofuscus*, n = 50), and spotted eagle owl (*Bubo africanus*, n = 5) (see Supplementary Table 2.2 for details). Additional parasite sequences collected from raptor species were downloaded from GenBank (March, 2020) and MalAvi (Bensch et al, 2009; Supplementary Table 2.3 & 2.4). Twenty four *Leucocytozoon* sequences were included from MalAvi. Species included: long-eared owl (*Asio otus*, n = 3), great horned owl (*Bubo*

virginianus n = 6), Sjöstedt's barred owlet (*Glaucidium sjostedti*, n = 1), northern saw-whet owl (*Aegolius acadicus*, n = 1), Eurasian scops owl (*Otus scops*, n = 2), western marsh harrier (*Circus aeruginosa*, n = 1), barn owl (*Tyto alba*, n = 1), cinereous vulture (*Aegypius monachus*, n = 1), black kite (*Milvus migrans*, n = 2), Eurasian sparrowhawk (*Accipiter nisus*, n = 4), Levant sparrowhawk (*Accipiter brevipes* n = 2), France's sparrowhawk (*Accipiter francesiae*, n = 1) and common buzzard (*Buteo buteo*, n = 1). Two sequences from GenBank included the northern saw-whet owl (*Aegolius acadicus*, n = 1) and the American kestrel (*Falco sparverius*, n = 1).

The *Haemoproteus* data set included 21 sequences downloaded from GenBank (Supplementary Table 2.3). These sequences included additional Amur falcon (n = 2) sequences as well as sequences collected from American kestrel (*Falco sparverius*, n = 3), Eurasian eagle-owl (*Bubo bubo*, n = 1) Eurasian sparrowhawk (*Accipiter nisus*, n = 6), red-throated caracara (*Ibycter americanus*, n = 1), common buzzard (*Buteo buteo*, n = 1), Japanese sparrowhawk (*Accipter gularis*, n = 1), common kestrel (*Falco tinniculus*, n = 1), spotted owlet (*Athena brama*, n=1), barn owl (*Tyto alba*, n = 4) and the lesser kestrel (*Falco naumanni*, n = 1). Nine sequences were downloaded from MalAvi (Supplementary Table 2.4). These sequences included the black kite (*Milvus migrans*, n = 3), Amur falcon (n = 2), Eurasian eagle-owl (*Bubo bubo*, n = 3), barn owl (*Tyto alba*, n = 1), and lesser kestrel (*Falco naumanni*, n = 2). *Plasmodium* was not successfully sequenced (see results) and so phylogenetic analyses were not performed.

2.2.2 DNA extraction and amplification

The NucleoSpin® Tissue kit (Macherey-Nagel, Düren, Germany) was used for all DNA extractions. DNA was extracted from muscle tissue and blood stored on FTA cards (FTA® Elute cards, Whatman, Maidstone, UK) using the standard protocol for animal tissue. The protocol was modified to improve DNA yield by incubating the samples with Proteinase *k* and lysis buffer at 56 ºC for 24 hours in a shaking water bath. The lysate was incubated for 1 hour at 70 º C and the elution buffer (BE) was also pre-warmed to 70 ºC before use.

The cytochrome *b* gene was amplified from the parasite DNA using a nested PCR method. The primers HaemNFI/HaemNR3 were used for the first amplification, which were designed to detect DNA from species of *Plasmodium Haemoproteus*, and *Leucocytozoon* (Hellgren et al, 2004a; Sambon, 1908). The second amplification step used primers HaemF/HaemR2 (Bensch et al, 2000b) and HaemFL/HaemR2L (Hellgren et al, 2004a) to

amplify species of *Haemoproteus*/*Plasmodium* and *Leucocytozoon*, respectively (Table 2.1). This nested PCR method detects Low-level infections.

PCR reactions consisted of 5µl of OneTaq 2x master mix (New England Biolabs, South Africa), 0.2 µM of each primer, 0.2 µl of bovine serum albumin (BSA), 2µl of template DNA, purified water was added to each reaction to make up a reaction volume of 10µl. The PCR product from amplification using HaemNFI/HaemNR3 (1ul) was used as template DNA for the second round of amplification using HaemF/HaemR2 and HaemFL/HaemR2L primers. The thermocycler conditions were as follows: initial denaturation at 94ºC for 2 minutes, denaturation at 94ºC for 30 seconds, annealing at 50ºC for 30 seconds, extension at 68ºC for 60 seconds and final extension at 68ºC for 5 minutes. No template controls were included in all PCR reactions. After an initial round of PCRs, all negative samples were rescreened to ensure accuracy. In this case, the nested PCR method was modified with the following: 1 µl of PCR product was used instead of the 0.5 µl used (from the first reaction consisting of HaemNFI/HaemNR3 primers).

All amplicons were sent to the Central Analytical Facilities (Stellenbosch University, South Africa) for Sanger sequencing. DNA sequencing was done using the BigDye Terminator V3.1 sequencing kit (Applied Biosystems) using the manufacturer's protocol with slight modifications. Electrophoresis is performed on an ABI3730xl using a 50cm capillary array and POP7 (all supplied by Applied Biosystems).

Table 2.1: Details of the three primer pairs used to amplify the cytochrome *b* gene of *Plasmodium*, *Haemoproteus*, and *Leucocytozoon* haemosporidian parasites infecting birds.

Genus	**Name**	**Primer sequence (5' – 3')**	**Product size (bp)**	**Reference**
Plasmodium	HaemF	ATGGTGCTTTCGATATATGCATG	480	Bensch et al, 2000; Hellgren et al, 2004
	HeamR2	GCATTATCTGGATGTGATATGGT	480	
Haemoproteus	HaemFL	ATGGTGCTTTCGATATATGCATG	480	Bensch et al, 2000; Hellgren et al, 2004
	HeamR2	GCATTATCTGGATGTGATATGGT	480	
Leucocytozoon	HaemFL	ATGGTGTTTTAGATACTTACATT	478	Hellgren et al, 2004
	HeamRL2	CATTATCTGGATGAGATAATGGIGC	478	

2.2.3 Phylogenetic analyses

Sequences for each parasite genus were aligned independently using Clustal X (Thompson et al, 1997) in Bioedit V7.0 (Hall, 2004). All alignments were manually optimised to ensure homology. The total number of nucleotide sites, variable and parsimony-informative sites, as well consistency index (CI), retention index (RI), and frequencies of nucleotides in each alignment as well as Nei's genetic distance, were determined in Mega (Kumar et al, 1994). Phylogenies were inferred using both maximum likelihood and Bayesian methods. JmodelTest 2 XSEDE v2.1.6 (Posada, 2009) run on the CIPRES Science Gateway (CSG; Miller et al. 2011) was used to estimate the best-fit substitution model for each data set. The program Garli 2.01 (Bazinet et al, 2014) was used to conduct maximum likelihood analyses. To estimate branch support 1000 bootstrap replicates were performed on the CSG server. Bayesian inference was conducted using Mr.Bayes V3.2.7a (Ronquist et al, 2012). To ensure convergence of the MCMC chains, two separate analyses were run, each for 20 million generations with trees sampled every 300 generation. The convergence of MCMC chains was accessed using Tracer V7.2.1 (Rambaut et al, 2018). Convergence was achieved when effective sample size (ESS) values were all >200. The first 25% of trees in the tree files were removed as burn-in. Majority rule (50%) consensus trees were estimated in Phylip 3.66 (Felsenstein, 2004). All trees were viewed and edited in FigTree V1.4.3 (Rambaut, 2012). All trees were midpoint rooted. Lineages with high to moderate bootstrap and posterior probability support (≥ 60% bootstrap values and ≥ 0.60 posterior probability values) were annotated onto the most likely tree. In addition to maximum likelihood and Bayesian methods, phylogenetic associations were also reconstructed using a network approach. A minimum spanning network was created in Popart v1.7 (Bandelt et al, 1999) for both the *Haemoproteus* and *Leucocytozoon* data sets.

2.3 Results

Three hundred and thirty-eight specimens were screened for avian malaria using the nested PCR method. A total of 63 positive *Haemoproteus* infections were recorded. Interestingly the majority of the infections (n = 60) were recorded from the Amur falcons. Of the 60 positives, two could not be sequenced and were not included in phylogenetic analysis. Only two infections were recorded from spotted eagle owls and a single infection recorded from a white-backed vulture. Six *Leucocytozoon* infections were recorded. Again, the majority of infections were recorded from the Amur falcon (n = 5). A single positive *Plasmodium* infection was recorded in the Amur falcon. Unfortunately, this amplicon could not be

successfully sequenced despite multiple attempts. Co-infection occurred at a very low frequency with only three co-infections observed: two in Amur falcon and one in spotted eagle owl (Table 2.2).

Table 2.2: Summary of haemosporidian infections recovered using the nested PCR method.

Host species	**Sample size**	***Plasmodium***	***Haemoproteus***	***Leucocytozoon***	**Co-infections**
Amur falcon (*Falco amurensis*)	178	1	60	5	2
Jackal buzzard (*Buteo rufofuscus*)	50	0	0	0	0
Cape vulture (*Gyps coprotheres*)	73	0	0	0	0
Spotted eagle-owl (*Bubo africanis*)	5	0	2	1	1
White-backed vulture (*Gyps africanus*)	111	0	1	0	0
Crowned eagle (*Stephanoaetus coronatus*)	3	0	0	0	0

2.4 Alignments

Sequences were obtained for 58 *Haemoproteus* and six *Leucocytozoon* infections. The single *Plasmodium* infection did not return a good quality sequence and no further phylogenetic analyses were conducted for this parasite genus. The final *Haemoproteus* alignment included 81 taxa, this included sequence data generated in this study as well as data downloaded from public databases. The final alignment was 480 bp in length and included 90 variable sites, 52 of which are parsimony informative (Table 2.3). The final *Leucocytozoon* alignment included 34 taxa and was 470 bp in length and included 63 variable sites, 21 of which are parsimony informative (Table 2.3). The final alignments contained no insertions, deletions, or stop codons. The nucleotide compositions of all the sequences were heavily biased toward A and T nucleotides. This is not unusual as avian haemosporidians are known to be AT-rich (Videvall, 2018). The topologies recovered by maximum likelihood and Bayesian analyses were visually compared and no instances of conflict were found. Maximum likelihood bootstrap values and posterior probabilities were annotated onto the midpoint rooted most likely trees for both data sets. The retention index (RI) and consistency (CI) index values indicate that some homoplasious characters are present in both data sets, justifying the use of model-based methods of phylogeny construction.

Table 2.3: Patterns of sequence variability of the mtDNA cytochrome *b* alignments for each parasite genus. The total taxa, total number of nucleotide sites, variable and parsimony-informative sites, consistency index (CI), retention index (RI), nucleotide frequencies, and best-fit nucleotide substitution model are given for each alignment.

Genus	**Total taxa**	**Total sites**	**Variable sites**	**Parsimony inf. Sites**	**CI**	**RI**	**Nucleotide frequencies**				**Best fit model**
							%A	**%T**	**%C**	**%G**	
Haemoproteus	81	480	343	96	0.65	0.91	29.4	42.8	14.0 %	13.8	GTR+I+G
Leucocytozoon	34	470	183	151	0.66	0.90	29.5	42.9	14.0	13.5	GTR+I+G

2.5 Phylogenetic analyses

2.5.1 *Haemoproteus*

The *Haemoproteus* maximum likelihood tree recovered two main lineages (Figure 2.1). A well-supported (95.1/1.0) lineage contained parasites isolated from five different raptor hosts however no parasites infecting Amur falcons were placed in this lineage (Figure 2.1). The remaining taxa all belong to the same grouping. This grouping is split into three lineages. One lineage contains parasite sequences from three black kites and is well supported (99.6/1.0). The second lineage contained parasites isolated from three Eurasian eagle-owls as well as one parasite lineage from a common buzzard. The third lineage contains all the parasite sequences isolated from the Amur falcon and lesser kestrel. This Amur falcon parasite lineage is further subdivided into two clades. The first contained parasite sequences amplified from 49 Amur falcons while the second lineage contained parasite sequences from eleven Amur falcons and included sequences from two lesser kestrels. This phylogenetic pattern was confirmed in the network analysis (Figure 2.2) which showed a distinct starburst pattern. Fifteen parasite alleles were recovered from the Amur falcon. Of those 14 were found only in the Amur falcon. Thirty-three Amur falcons were infected by parasite allele 4. The average genetic distance of *Haemoproteus* sequences recovered was 0.01. The lineage containing the 48 Amur falcon parasites (Figure 2.1) had an average Nei's genetic distance of 0.01 while the second lineage that containing the 11 parasite sequences from the Amur falcon and the four parasite sequences from the lesser kestrels (Figure 2.1) had an average genetic distance of 0.02.

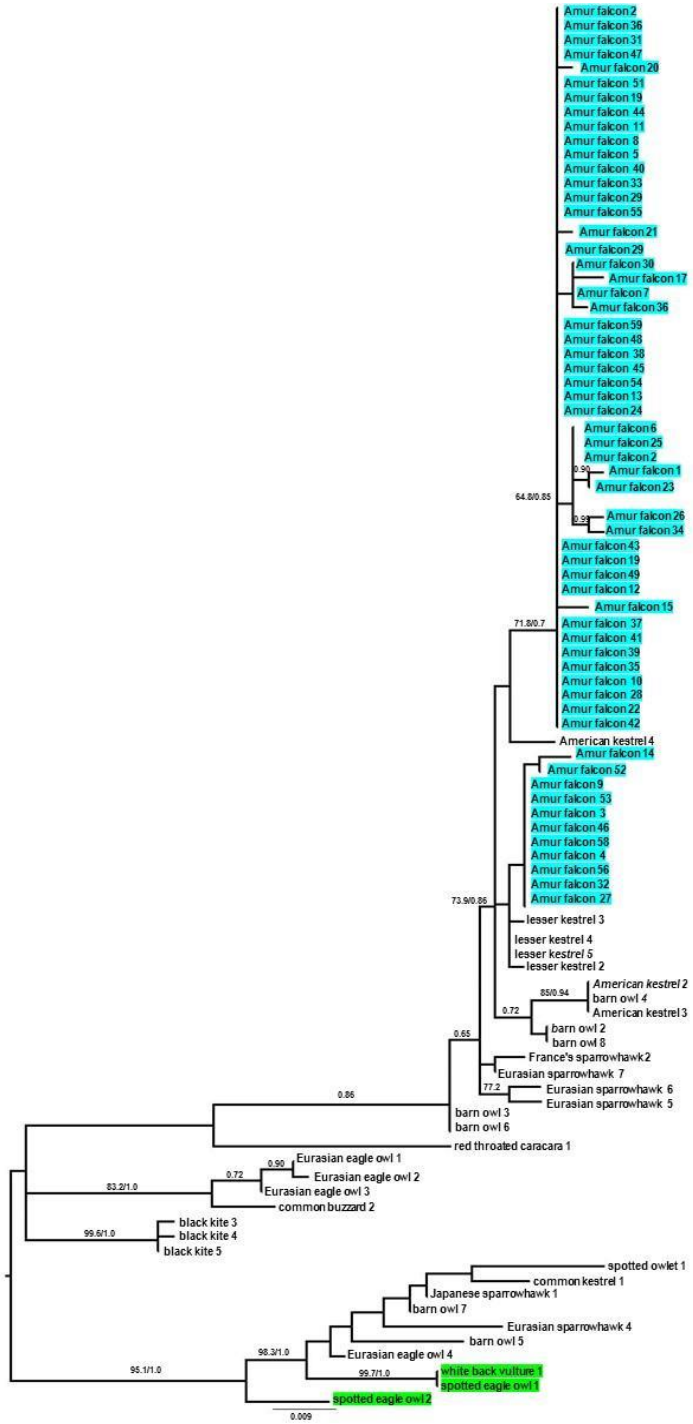

Figure 2.1**:** Maximum likelihood phylogeny of avian *Haemoproteus* sequences. The phylogeny is mid-point rooted with bootstrap values ≤ 60 % and posterior probability values ≤ 0.60 are annotated onto branches. Sequences are labelled by host. Parasite sequences isolated from the Amur falcon are highlighted in blue. Parasite sequences isolated from other South African raptors are highlighted in green. For details of each specimen please see Table 2, 3 and 4 in the supplementary information.

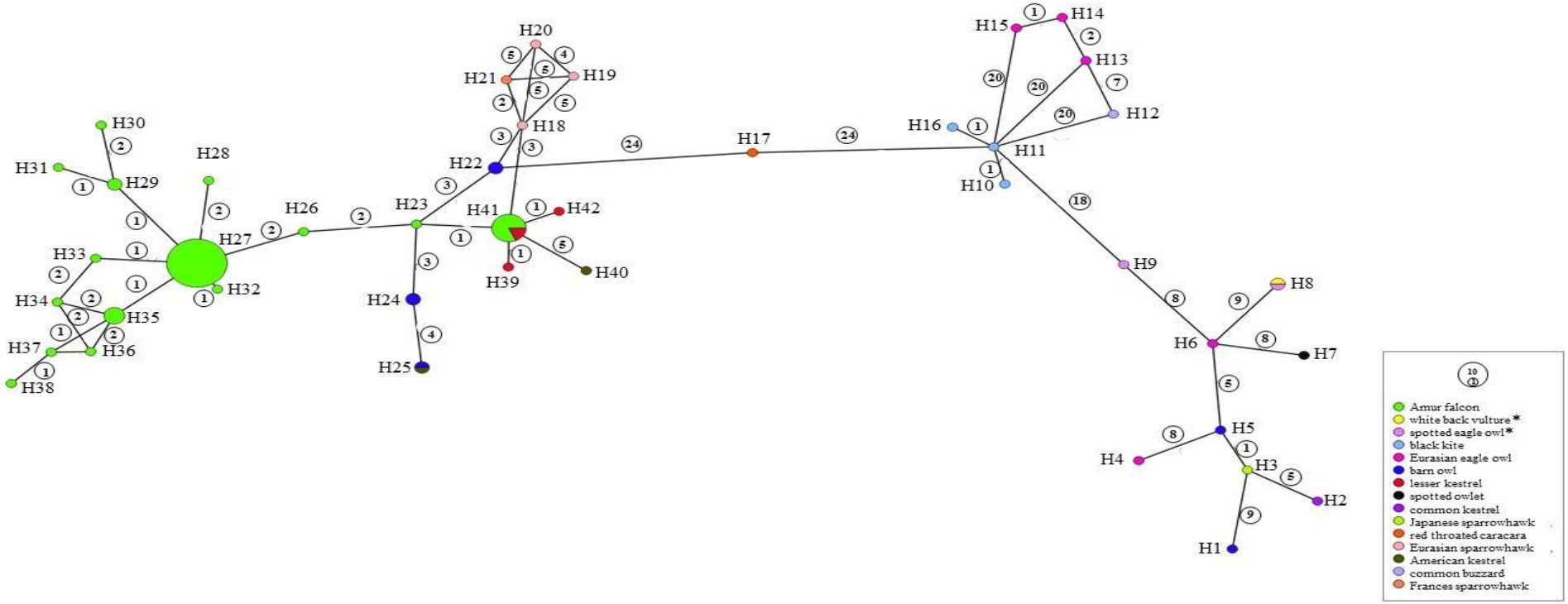

Figure 2.2: Allele network including *Haemoproteus* sequences analysed in the present study. Haplotypes have been coloured according to host species. Parasite sequences from other South African birds are indicated by *. Circled numbers indicate the number of mutational steps separating cytochrome *b* haplotypes.

2.5.2 Leucocytozoon

The *Leucocytozoon* maximum likelihood tree showed two well-supported main lineages (Figure 2.3). One lineage (100/1.0) contained three clades. The first clade was well supported (100/1.0) and contained *Leucocytozoon* parasites isolated from only the Eurasian sparrowhawk. The second clade contained parasites isolated from two hosts - the Levant sparrowhawk, and France's sparrowhawk. The third clade (94.9/0.99) contained a mixture of parasites amplified from the Eurasian sparrowhawk, common buzzard, and black kite. The second main lineage (100/0.1) is also split into three clades. The first clade contained only a single sequence, from a black kite host. Amur falcon 1, Amur falcon 60, Amur falcon 16, and Amur falcon 54 formed a separate clade, together with a sequence recovered from an American kestrel. Another clade (67.8/0.96) contained sequences isolated from Amur falcon 55 as well as a barn owl, Cinereous vulture, and Western marsh harrier. The last clade in this lineage contained only a great horned owl. The third lineage contained two clades with parasites from single hosts, a great horned owl, and a long-eared owl. Parasites from Eurasian scops owl formed a well-supported (87.2/1.0) clade. Two parasite sequences from the long-eared owl host formed a well-supported (98.2/1.0) clade with a sequence from great horned owl. Parasites from the spotted eagle owl, boreal owl, great horned owl, northern saw-whet owl, and the Sjöstedt's owlet formed a clade.

The phylogenetic associations recovered by the maximum likelihood and Bayesian methods were confirmed by the network analysis of *Leucocytozoon* sequences (Figure 2.4). Two parasite alleles were found in the Amur falcons however both were shared with other species. Three Amur falcons shared an allele with an American kestrel (allele 2; Figure 2.4). One Amur falcon shared an allele with a western marsh harrier (allele 1; Figure 2.4). There are only two mutations between shared allele 2 and barn owl 1 and one mutation between shared allele 1 and barn owl 1. The different species of sparrowhawks (*Accipter nisus*, *Accipter brevipes* and *Accipter francesiae*) formed their own lineage characterised by starburst pattern. This distinctive pattern was also seen when the *Leucocytozoon* sequences from different species of owls (*Asio otus*, *Aegolius acadicus*, *Glaucidium sjostedti* and *Aegolius funereus*) are considered. The Nei's average genetic distance of *Leucocytozoon* sequences was1.93

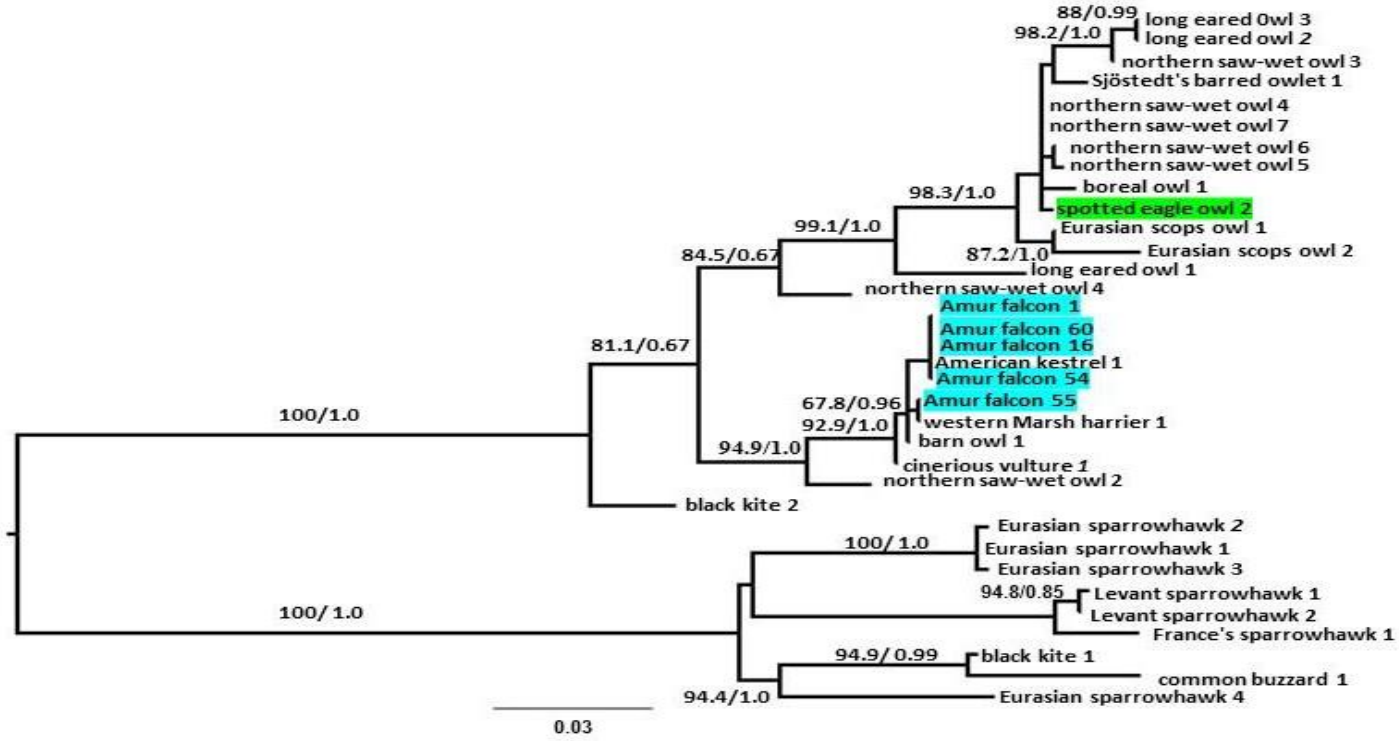

Figure 2.3: Maximum likelihood phylogeny of avian *Leucocytozoon* sequences. The phylogeny is mid-point rooted with bootstrap values ≤ 60 % and posterior probability values ≤ 0.60 are annotated onto branches. Sequences are labelled by host. Parasite sequences isolated from the Amur falcon are highlighted in blue. Parasite sequences isolated from other South African raptors are highlighted in green. For details of each sequence included refer to Table 2, 3, 4 and 5 in the appendix.

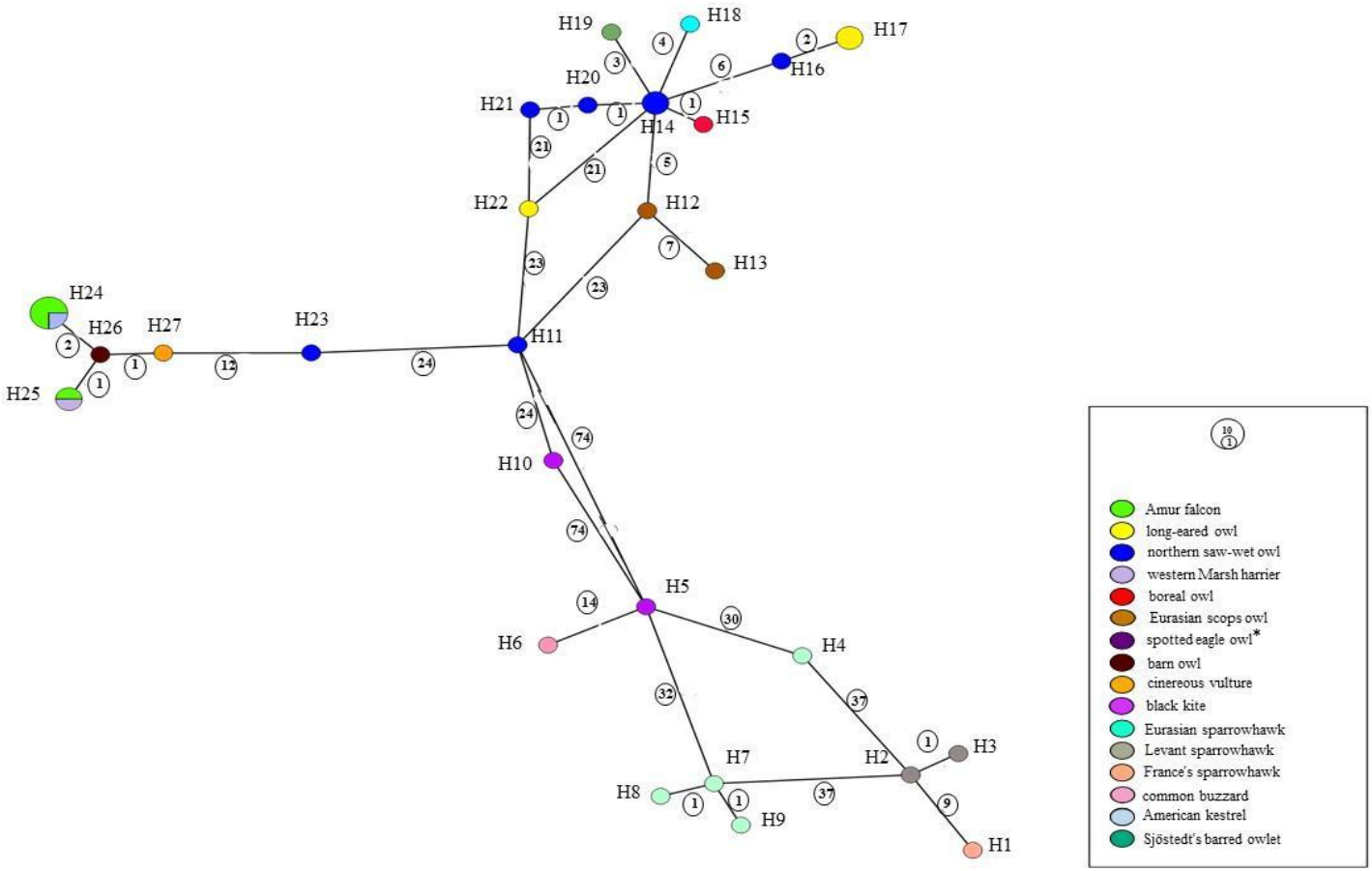

Figure 2.4**:** Allele network including *Leucocytozoon* sequences analysed in the present study. Haplotypes have been coloured according to host species. Parasite sequences from other South African birds are indicated by *. Circled numbers indicate the number of mutational steps separating cytochrome *b* haplotypes.

2.6 Discussion

This study aimed to test for the presence of the parasites belonging to *Plasmodium, Haemoproteus,* and *Leucocytozoon* in a large sample of Amur falcon. The Amur falcon is an intercontinental migratory raptor and by including parasite sequences isolated from other raptors found in South Africa this study also aimed to determine the impact of migration on local populations of parasites infecting raptors. The few studies that have examined raptor parasite prevalence have shown that *Plasmodium* infection occurs rarely, unlike what has been observed in passerine hosts (Nourani et al, 2020). This observation was supported in this study with only a single *Plasmodium* infection recovered. Previous studies have recovered *Leucocytozoon* and *Haemoproteus* as the most prevalent haemosporidian infections in raptors (Coeurdassier et al, 2021; Nourani et al, 2020; Pornpanom et al, 2019). In this study *Haemoproteus* was the most prevalent infection with 15% of raptors tested being infected. *Haemoproteus* was also the most prevalent parasite infecting the Amur falcon with 34% of birds screened infected with this parasite, and only 3% infected with *Leucocytozoon*. A study done on 13 species of raptors showed similarly lower infection rate of *Leucocytozoon* as the Amur falcons (Nourani et al, 2020). However, the high prevalence of *Haemoproteus* infection in the Amur falcon was surprising and a key finding of this study. Another study done on the Eleonora's falcon, also a long-distance migrant and member of the genus *Falco,* showed a much lower *Haemoproteus* infection rate of 9.5% but similar (2.4%) *Leucocytozoon* infection rate (Gutiérrez-López et al, 2015). The other large samples of South African raptors tested in this study all recovered much lower rates of infection. The reason for the exceptionally high *Haemoproteus* infection rate in the Amur falcon is not clear however , in another study done on influenza A viruses, the Newcastle Amur falcons showed a high infection rate of 42% (El Zowalaty et al, 2021). Additional research is needed on this topic.

Co-infection by haemosporidian parasites has been of research interest in the literature, because survival of the host with multiple infections has been shown to be decreased (Pigeault et al, 2018). There is evidence that competition between parasites may increase virulence in the host (Bell et al, 2006), however, there are also examples where virulence has decreased (Gower & Webster, 2005). Therefore there is limited evidence to predict if co-infection by two or more of the haemosporidian parasites will have an increased or a decreased effect in virulence in the host (Bull, 1994). If a host is infected with one lineage or species of parasite they could be more susceptible to being infected by another parasite as the immune system may be under pressure (De Roode et al, 2005; Palinauskas et al, 2011; Zehtindjiev et al, 2008). This hypothesis predicts that co-infection will occur at a high frequency which has been supported

by studies that have shown a high frequency of co-infections by haemosporidian parasites (Elikwo et al, 2020; Galen et al, 2019; Palinauskas et al, 2011; Valkiunas, 2004). However, in this study, this hypothesis was not supported as only two Amur falcons and one spotted eagle owl had co-infections. This may be due to the fact that the studies with high frequencies of co-infections were done on passerines while few studies on raptors have reported high frequencies of co-infection (Ciloglu et al, 2016). A study done on 167 owls (including the species *Glaucidium cuculoids*, *Tyto alba*, *Otus lettia*, *Athene brama*, *Bubo sumatranus*, *Ketupa ketupa*, *Ninox scutulata*, *Strix leptorammica*, *Philodilus badius*, *Otus sunia*, *Asio flammeus* and *Bubo nipalensis*) showed only one co-infection (Pornpanom et al, 2019). Similarly in a study of 11 common buzzards (*Buteo buteo*) only one co-infection was recovered (Shokrani et al, 2021). A study in Thailand (Pornpanom et al, 2021) found no co-infections in 22 different raptor species tested (a total of 198 birds). These findings collaborate the results from this study suggesting that unlike in passerine species co-infections occur rarely in raptors.

Host specificity of blood parasites is a key factor in understanding the pathogenicity of these haemosporidian parasites. Literature has shown that species belonging to *Plasmodium* are more generalist (Clark et al, 2014; Hellgren et al, 2013; Reeves et al, 2015; Svensson-Coelho et al, 2013). In contrast, parasite species belonging to *Haemoproteus* have shown higher levels of host specificity (Atkinson & Van Riper III, 1991). *Leucocytozoon* has been documented to infect a broad range of avian families and are not generally host specific (Atkinson & Van Riper III, 1991b). If a parasite is associated with a single host this would indicate that the host and parasite have co-evolved (Hoberg et al, 1997). Host switching may impact the virulence of the parasites (Toft & Karter, 1990). The native theory implies that a parasite will be more virulent when infecting a species that the parasite has no coevolutionary history with (Lymbery et al, 2014). There are a number of factors that impact virulence in host switching, these factors include intrahost competition, the host's immune system and parasite genetic recombination (Rigaud et al, 2010). In this study, our data provides evidence supporting species belonging to *Haemoproteus* as being more host specific. In particular, a single lineage of *Haemoproteus* was found only in Amur falcon hosts. Interestingly, the high amount of genetic diversity present in *Haemoproteus* isolated from the Amur falcons suggests that multiple *Haemoproteus* species may have co-evolved with these hosts. Given that this study was only based on molecular data, species delimitation will need to be confirmed by a taxonomist. Even when multiple hosts were found to be infected by the same lineage of *Haemoproteus,* the taxonomy of the host seems to play an important role. For example, the same lineage of *Haemoproteus* was found to infect both Amur falcon and lesser kestrel – these

species both belong to the genus *Falco*. In contrast the data presented in this study could not find any evidence to suggest that *Leucocytozoon* parasites are host specific, with parasite lineages infecting the Amur falcon also found in other hosts. This study thus suggests that *Leucocytozoon* is more generalist, and that host switching occurs much more frequently. This is an important consideration given that the Amur falcon is a long-distance migrant. Any *Leucocytozoon* parasites carried by these birds have a high chance of being transmitted to local bird populations.

Long distance migrants can extend a parasite's geographical range extensively (Hellgren et al, 2007; Ricklefs et al, 2017). Migratory birds may introduce new lineages of parasites into local populations however little is understood about the transferral of parasites from migratory birds to local populations (Ricklefs et al, 2017). Parasites introduced to South Africa by migrating Amur falcons may impact local populations of parasites and hosts. Despite including large samples of other raptor species from South Africa and specifically KwaZulu-Natal, this study found no evidence that of *Haemoproteus* and *Leucocytozoon* species found in the Amur falcon were shared with other South African raptors. Additional samples of birds that occur sympatrically with the Amur falcon would need to be analysed to confirm this finding.

In conclusion this study showed that Leucocytozoon was not host specific while *Haemoproteus* was host specific in the Amur falcon. Haemoproteus lineages recovered in this study infected both the Amur falcons and the Lesser kestrels indicating that this lineage may impact local breeding populations. *Leucocytozoon* may be more dangerous, due to it not being host specific, as the Amur falcon may introduce new lineages to local populations however there is no evidence that this has occurred. Future studies with a large dataset of *Plasmodium* parasites in the Amur falcon could provide insight into the host specificity of the *Plasmodium* genus in raptors.

No other South African falcons were included in this study which may have provided key information on host specifity of the parasites. Future studies including KZN falcons such as the Lannar falcon (*Falco biarmicus*) and the rock kestrel (*Falco rupicolus*) could provide key comparisons to the Amur falcon in terms of host specifity.

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CHAPTER 3: Genetic diversity and population structure of Amur falcon (*Falco amurensis*) in KwaZulu-Natal, South Africa

Abstract

There is evidence to suggest that migration impacts genetic diversity and population structure in bird populations. The high rates of gene flow associated with highly mobile species suggests that migration may lead to genetically homogenise populations leading to low levels of genetic diversity and shallow population structure. In contrast, natal philopatry may actually lead to increased genetic structure even in highly mobile species. The Amur falcon (*Falco amurensis*) is a small migratory raptor that breeds in Eastern Asia before migrating to Southern Africa over winter. Eight microsatellite loci were amplified from 178 Amur falcons collected in KwaZulu-Natal, South Africa to measure the genetic diversity and population structure present. Observed heterozygosity estimates (0.67) and low inbreeding (0.14) indicate high levels of genetic diversity present in the population. Bayesian assignment tests recovered a very shallow population structure.. This study provides baseline data for studies investigating the impact of migration on genetic diversity and population structure in a raptor.

3.1 Introduction

Migration can refer to the movement of a species or individual without any genetic exchange however in a population context migration refers to gene flow that occurs due to migration (Slarkin, 1985). Migratory connectivity is a key influence to population structure as it defines the movement and interaction with other individuals which may result in gene flow (Webster et al, 2002). High migration connectivity is often a result of little interaction with other individuals and a group of individuals following the same migration route at the same time. Low migratory connectivity is often due to separation of individuals on a migratory route. Strong migratory connectivity may result in population structure (Webster & Marra, 2005). Recent phylogeny and geographic studies have revealed greater population structure and genetic diversity in migratory versus nonmigratory lineages (Rolland et al, 2014). (Vagrancy or the long-distance dispersal of species outside of their known range can allow individuals to respond to potential inbreeding variable habitat and population density (Shields, 1982). In birds, vagrancy is more pronounced in long-distance migrants where meteorological conditions such as storms or wind could cause birds to drift past or overshoot their anticipated destinations

during seasonal migration (Newton 2008). Vagrant individuals could establish viable allopatric breeding populations leading to genetic divergence and population structure Alternatively, migration may homogenise populations leading to very shallow genetic structure even over large geographic distances (Bensch et al, 1999).

The Amur falcon (*Falco amurensis*) undertakes one of the longest migrations of any raptor in the world. The species breeds in south-eastern Siberia and Northern China before travelling 14 500 km across India and the Arabian sea to overwinter in Southern and East Africa (Symes & Woodborne, 2010). In addition to anthropogenic threats such as hunting - between 120 000 and 140 000 Amur falcons from a single roosting site were harvested over a span of two weeks in 2012 (Bouwman et al, 2012; Dalvi et al, 2013), meteorological events may also have a severe effect on populations. In 2019, 700 falcons died in a hailstorm in Mooi River and over 1000 were injured and died in a second hailstorm in Newcastle, South Africa (Jones 2019). What effect these bottleneck events have on the genetic diversity of Amur falcon populations is unknown.

Using samples collected from falcons killed in the 2019 hailstorms in KwaZulu-Natal, South Africa this study aims to assess the genetic variation and population structure present in the Amur falcon. Using genetic data I also test for signatures of recent population bottlenecks which may be attributed to increased hunting pressure on these birds.

3.2 Materials and methods

3.2.1 Sampling

A total of 178 Amur falcon liver samples were obtained from the Durban Natural Science Museum for genetic analyses (Supplementary Table 3.1). These samples were collected from birds killed in hail storms at two localities in 2019: Mooi River (n = 50) and Newcastle (n=128), KwaZulu-Natal, South Africa. Ethical approval for the study was given by the Animal Research Ethics Committee (Reference number AREC/022/020).

3.2.2 DNA extractions and microsatellite amplification

DNA extractions were performed using the NucleoSpin® Tissue kit (Macherey-Nagel, Separation, South Africa). Liver extractions followed the standard protocol for animal tissue. The protocol was modified by incubation of the samples with Proteinase *k* and lysis buffer at

56 ºC for 24 hours in a shaking water bath. The lysate was incubated for 1 hour at 70 º C. The BE buffer was pre-warmed to 70 ºC before use. No microsatellite loci primers have been designed specifically for use in the Amur falcon therefore twenty-two microsatellite loci previously published for use on other species (*Falco vespertinus, Buteo buteo, Buteo swainsoni*) were screened for utility in the Amur falcon. Ten of the loci screened were designed for the red-footed falcon (Magonyi et al, 2019), seven of the loci were designed for the common buzzard (Johnson et al, 2005) and five were designed for Swainson's hawk (Hull et al, 2007). Based on amplification success and variation, eight microsatellite loci were selected for amplification in the Amur falcon. The eight microsatellite loci were amplified in four multiplex reactions (Table 3.1) using Tempase^{TM} Fast Multiplex PCR kit (Ampliqon, Denver). The reactions contained 5 µl Tempase multiplex mix, 0.2 µl of each primer and 0.5 µl of DNA. Total volume of each reaction was made up to a final volume of 10 µl with water. The thermocycler conditions were as follows: 95º C for 15 minutes for initial denaturation, 35 cycles at 95º C for 60 seconds, 60º C for 1 minute, 72º C for 60 seconds followed by a final extension step at 72º C for 5 minutes. No template controls were included to check for the contamination of reagents. All PCR products were run on a 1% agarose gel. All positive products that were successfully amplified were sent to the Central Analytical Facility (CAF) at Stellenbosch University, South Africa for fragment analysis. The 3500xl Genetic Analyzer (Thermo fisher scientific, United States) is used to run fragment analyses. Genotypes were scored using Genemarker v2.4.0 (Softgenetics) with a size standard of GS500_old_1 (Supplementary Table 3.5). To ensure the accuracy of the results, 20% of all samples were genotyped twice and scores compared.

Table 3.1: Details of microsatellite loci used in the present study to genotype Amur falcon (*Falco amurensis*).

Locus	Primer sequence	Motif	Annealing temperature (ºC)	Label	Allele size range (bp)	Multiplex reaction	Reference
FalVes13	F: AACAAGTGCTGTTCCTGATG R: TGTGCACTTCTAATGCTGGTC	(ATT)19	60	HEX	97-166	Multiplex 1	Magonyi et al, 2019
FalVes28	F: CACATTCCTCGAGCAGACAC R: AGCAGGACTCTTTCCAGTGAG	(TATC)22	60	HEX	199-325	Multiplex 1	Magonyi et al., 2019
FalVes38	F: ACAAGCCGAAATGAAGCGAG R: GACAGTAGCGGCTGGTTTTC	(GAAA)9 & (AG)10	60	FAM	216-289	Multiplex 2	Magonyi et al., 2019
FalVes31	F: CCTCAGGAAACAAGTCTGGG R: TGTTAGCTGATGGCCACTTTTC	(GAAT)10	60	TET	108-144	Multiplex 2	Magonyi et al., 2019
FalVes43	F: TGTGGCTTTCGCATTTCTGG R: GTCATTTAGGCATTTCACTGCTG	(TATT)10	60	FAM	195-225	Multiplex 3	Magonyi et al., 2019
FalVes05	F: TCACAATGCCTTTAGACCTCTG R:AGGATGCAACTTTGACATTTTTGG	(GATG)23	60	HEX	181-249	Multiplex 3	Magonyi et al., 2019
Bbu03	F: GATCAAAGTACTTGACAGTGTCC R: CAGGTACATGCGTACATACTTC	(GA)5	60	FAM	190-210	Multiplex 4	Johnson et al., 2005
FalVes26	F:TCCTGAGAGGCATAAACATTTTGG	(AC)17	60	TET	189-215	Multiplex 4	Magonyi et al., 2019

R: TATGCAGGAACCAACTCACG

3.3 Data analyses

3.3.1 Estimating genetic diversity

One of the limitations of using microsatellite primers designed for use in other species is the problem of null alleles (Hedgecock et al, 2004). Null alleles can inflate Fst values, biasing population genetic differentiation analyses. Locus Null allele frequencies were estimated using the expectation maximisation (EM) algorithm (Dempster et al, 1977) in FreeNA (Chapuis & Estoup, 2007). A paired t-test was run in Excel comparing uncorrected Fst values and corrected Fst estimated using the excluding Null alleles (ENA) algorithm, to determine if null alleles are affecting the population structure analyses. The polymorphic information content (PIC) of each locus was estimated in Cervus v3.0.7 (Kalinowski et al, 2007; Marshall et al, 1998). Loci with PIC values ranging from 0 to 0.29 are considered uninformative, 0.30 to 0.49 are moderately informative and PIC values above 0.50 are highly informative (Mateescu et al, 2005). Genetic diversity was evaluated by calculating observed heterozygosity, unbiased expected heterozygosity, the number of alleles and fixation index (F) in GeneAIEX v6.502 (Peakall & Smouse, 2012). Genepop v 4.2 (Raymond, 1995) was used to test for deviation from Hardy Weinberg equilibrium. Allelic richness and inbreeding coefficient (F_{IS}) was calculated using the programme FSTAT v2.9.3.2 (Goudet, 1995).

3.3.2 Population structure

Mantel tests were performed in GenAlEx (Peakall & Smouse, 2006) in order to determine if there was a correlation between pairwise geographical distance and pairwise genetic distance (Mantel, 1967). STRUCTURE v2.3.4 (Pritchard et al, 2010) was used to run Bayesian assignment tests. STRUCTURE runs using the admixture model with correlated allele frequencies were performed using 100 000 Markov chain Monte Carlo (MCMC) replicates and a burn-in of 10 000. The number of genetic clusters (K) ranged from 2 to 5. The optimal K (delta K) was estimated using the Evanno method (Evanno et al, 2005) and the Puechmaille method (Puechmaille, 2016) in the programme STRUCTURE harvester v0.6.94 (Earl & VonHoldt, 2012). Pophelper v2.3.1 (Francis, 2017) was used to create bar plots from STRUCTURE runs. Membership probabilities (Q-values) were estimated in ClumpAK (Kopelman et al, 2015).

Signals of population structure were also visualised using Principal Coordinate Analysis (PCoA) in GenAlEx. These analyses were performed using pairwise genetic

distances. PCoA uses multidimensional scaling to show similarity and clustering in a dataset (Zuur et al, 2007). Pairwise F_{ST} were estimated in FSTAT v2.9.3.2.

3.3.3 Population bottleneck and effective population size

BOTTLENECK v1.2.02 (Piry et al, 1999) was used to test for heterozygosity excess (Hx; Cornuet & Luikart, 1996). Two mutational models were used, the conservative stepwise mutational model (SMM) and the two-phase model (TPM). A 90% stepwise mutation model was used following recommendations in other studies (Cornuet & Luikart, 1996; Garza & Williamson, 2001) with a variance of 12 as suggested by the publisher of the programme (Piry et al, 1999). These models and parameters are chosen specifically to ensure that the multistep mutations that occur in natural populations are included in the analyses (Di Rienzo et al, 1994). Two statistical tests were used, the Wilcoxon sign-rank test and the sign test, to compare the expected equilibrium heterozygosity to the Hardy-Weinberg heterozygosity in order to determine heterozygosity excess (Piry et al, 1999).The Wilcoxin sign-rank test detects a recent decrease in effective population size (Ne) which ultimately results in a higher heterozygosity (Cornuet & Luikart, 1996). The sign test is used to determine heterozygosity excess or deficiency at a loci level (Cornuet & Luikart, 1996). The mode shift can differentiate between a bottlenecked or stable population. This statistical test can detect bottleneck events within a few generations. Therefore only recent bottleneck events can be determined (Luikart et al, 1998). Effective population size (Ne) was determined in NeEstimator V2.1 (Do et al, 2014) using two mating models (i.e. random and monogamous mating models).

3.4 Results

All eight loci were successfully amplified from 178 Amur falcons (Supplementary Table 3.1). The final data matrix included limited missing data. The missing data were included in the following loci: FalVes13 and Bbu03 (1.1% missing data each), FalVes28 (1.7% missing data), FalVes38 (4.5% missing data), FalVes31 (2.8% missing data), FalVes43 and FalVes05 (3.4% missing data each), FalVes26 (2.2%). The mean null allele frequency across all the 8 loci was only 6% (Table 3.2). FalVes43 and Bbu03 had the highest null allele frequencies of 19% and 15% respectively (Table 3.2), however the paired t-test indicated no significant difference between the uncorrected and corrected FST values suggesting that null alleles were not biasing analyses. The data for all the eight loci was used for subsequent analyses. Six of the eight loci were highly informative (PIC > 0.5). FalVes13 and Bbu03 had PIC values of 0.39 and 0.37 respectively. The mean PIC value across all loci was 0.74 which is considered highly

informative. FalVes13, FalVe38, FalVes31, and FalVes05 did not deviate from the Hardy-Weinberg equilibrium. FalVes28, FalVes43, Bbu03, and FalVes26 all significantly deviated from the Hardy-Weinberg equilibrium ($p < 0.05$).

3.4.1 Genetic diversity

The total number of alleles (A_T), null allele frequency (No), observed heterozygosity (Ho), and unbiased expected heterozygosity (uHe) are given in Table 3.2. Observed heterozygosity values for the different loci ranged from 0.24 to 0.93 with a mean of 0.67. The number of alleles (A_T) ranged from 6.5 for Bbu03 to 37 for FalVes28. The inbreeding coefficients ranged from -0.02 to 0.47. The mean inbreeding coefficient was 0.14. The fixation index (F) indicated that FalVes43 and Bbu03 had high levels of inbreeding (F>0.20). The mean F value for the eight loci was 0.14.

Table 3.2: Summary statistics for the eight microsatellite loci amplified from 178 Amur falcons (Falco amurensis). Number of alleles (AT), null allele frequency (No), uncorrected fixation index (FST A) and corrected fixation index (FSTB), observed heterozygosity (Ho), unbiased expected heterozygosity (uHe), deviation from Hardy-Weinberg (HWD), fixation index (F), inbreeding coefficient (FIS) and polymorphic information content (PIC) are shown.

Locus	**A_T**	**No**	**F_{ST}^A**	**F_{ST}^B**	**Ho**	**uHe**	**HWE**	**F**	**F_{IS}**	**PIC**
FalVes13	8	0.05	0.01	0.02	0.39	0.42	0.24	0.05	0.06	0.39
FalVes28	37	0.01	0.00	0.00	0.92	0.96	0.01	0.03	0.03	0.96
FalVes38	22.5	0.01	0.00	0.00	0.91	0.92	0.14	0.01	0.01	0.91
FalVes31	11	0.02	0.00	0.00	0.83	0.85	0.35	0.01	0.01	0.82
FalVes43	8.5	0.19	0.00	0.00	0.43	0.78	0.00	0.44	0.44	0.75
FalVes05	20.5	0.00	0.00	0.00	0.93	0.92	0.60	-0.02	-0.02	0.92
Bbu03	6.5	0.15	0.03	0.02	0.24	0.44	0.00	0.47	0.47	0.37
FalVes26	12.5	0.08	0.00	0.00	0.69	0.82	0.00	0.15	0.15	0.80
Mean	15.8	0.06	0.01	0.01	0.67	0.76	0.09	0.14	0.14	0.74

When individuals were grouped according to the two collection localities the number of alleles were 115 from Mooi River and 138 from Newcastle. Allelic richness was 14.22 in Mooi River and 14.19 in Newcastle. The observed heterozygosity was 0.66 for Mooi River and 0.68 for Newcastle and both populations deviated significantly from HWE (Table 3.3). The fixation index showed low inbreeding (F <0.20) in both populations.

Table 3.3: Genetic diversity estimates of the 178 Amur falcons (*Falco amurensis*). Number of individuals (N), total number of alleles (A_T), mean number of alleles ($\bar{A}$), allelic richness (A_R), observed heterozygosity (Ho), unbiased expected heterozygosity (uHe), fixation index (F), and inbreeding coefficient (F_{IS}) and allelic richness (A_R) are shown for each locality and overall.

Locality	**N**	**A_T**	**$\bar{A}$**	**A_R**	**Ho**	**uHe**	**F**	**F_{IS}**	**HWE**
Mooi River	50	115	14.38	14.22	0.66	0.79	0.17	0.12	0.03
Newcastle	128	138	17.25	14.19	0.68	0.75	0.11	0.09	0.30
Overall	178	153	19.13	14.68	0.67	0.76	0.14	0.14	0.09

3.4.2 Population structure

The Evanno method and the Puechmaille method recovered K = 4 as the number of optimal genetic clusters in the population (Supplementary Table 3.3). The graphs for K = 3 and K = 5 were also shown in Figure 1 for comparison. The membership coefficients (Q) were all very low indicating that there were no distinct geographical signals (Supplementary Table 3.4). All genetic clusters were seen in both Mooi River and Newcastle populations at similar frequencies (Figure 3.1). No correlation between geographical distance and pairwise genetic distance was found in the Mantel test (R = 0.095, P = 0.02; Supplementary Figure 3.1). The PCOA analyses supported the finding of the STRUCTURE analyses and showed no distinct clustering of individuals by locality. The lack of genetic differentiation between the birds sampled from Mooi River and Newcastle was also clear from pairwise FST values (Supplementary Table 3.2). The largest genetic distance was 0.01.

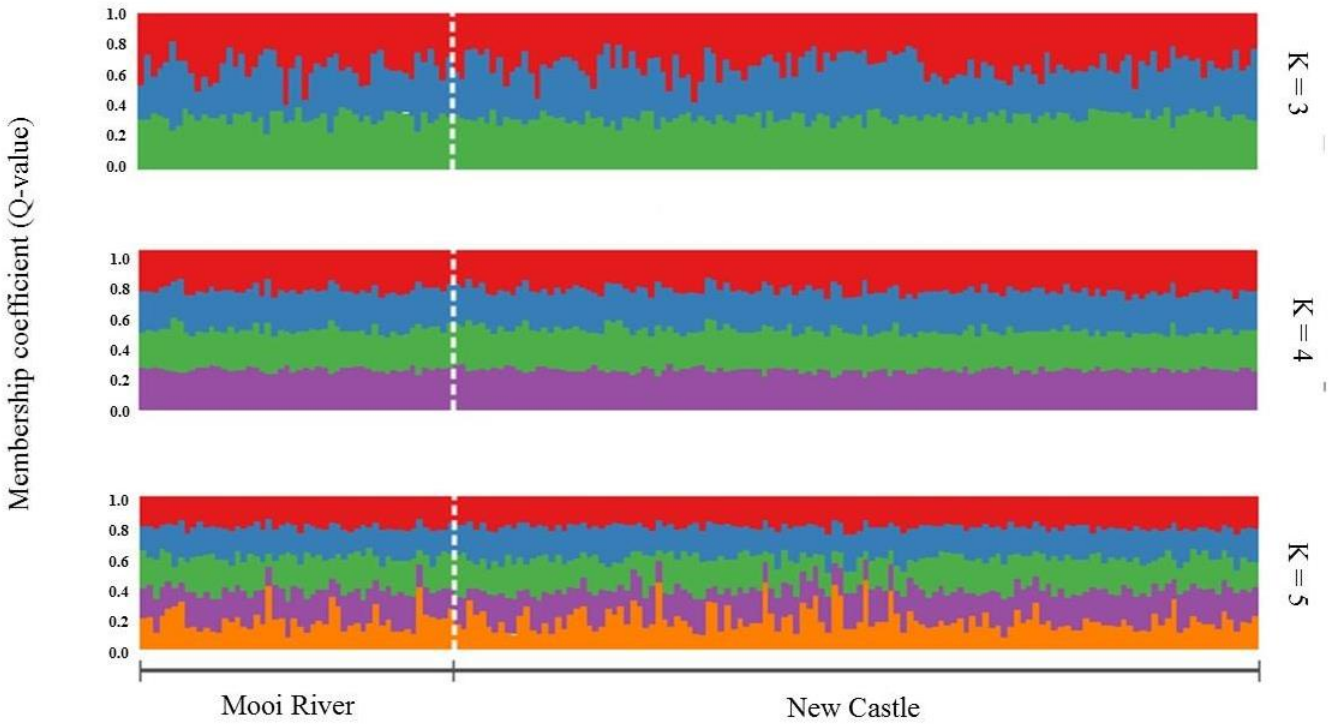

Figure 3.1: Structure bar plot of the 178 Amur falcon (Falco amurensis) sampled. Graphs for K = 3, K = 4, and K = 5 are shown however the optimal genetic cluster is K = 4. An individual is represented by each vertical line in the bar plot and is coloured according to the individual's estimated membership coefficient (Q) values (Supplementary Table 3.4).

3.4.3 Population bottleneck and effective population size

The SMM and TPM (with 90% stepwise mutation) models showed no sign of heterozygous excess which would indicate a recent bottleneck event (p-value > 0.05; Table 3.5). The Wilcoxin test showed significant heterozygosity deficiency in the SMM model for both populations as well as for the TPM model in the Newcastle population (p-values < 0.05). This was confirmed for the Newcastle population using the sign test with the SMM model. The heterozygous deficiency indicates that the Amur falcons are not in a mutational drift equilibrium (Table 3.4).

Table 3.4: Bottleneck results of the 178 Amur falcons (*Falco amurensis*) grouped by locality. Both the SMM and TPM models were used. Results from the Wilcoxon test and sign test are recorded below. Significant p values (p<0.5) are shown in bold.

Test	**Wilcoxon test**		**Wilcoxon test**		**Sign test**		**Sign test**		**Mode Shift**
	One tailed for Hx		One tailed for Hd		TPM		SMM		
	TPM	SMM	TPM	SMM	Hx:Hd	p	Hx:Hd	p	
Mooi River	0.96	0.99	0.10	**0.01**	4.73	0.19	4.76	0.05	No
Newcastle	0.97	1.00	**0.04**	**0.01**	4.00	0.15	4.82	**0.01**	No

The effective population size (Ne) was estimated using two models, the random mating model and the monogamous model. The random mating model estimated Ne = 430.4 individuals (CI: 215.80 ; 2756.00) The monogamous model estimated Ne = 860.901 individuals (CI: 432.80 ; 5467.70). This is much lower than that reported number of Amur falcons in a census conducted in South Africa in 2009 which approximated 111 219 individuals. This may be due to the Census including non-breeding individuals such as Juveniles. Therefore Ne is expected to be much smaller than the census size.

3.5 Discussion

This is the first study to examine the genetic diversity of the Amur falcon to date. Eight microsatellite markers previously designed for the red footed falcon (*Falco vespertinus*; (Magonyi et al, 2019) and the common buzzard (*Buteo buteo;* Johnson et al, 2005) were successfully amplified from the 178 Amur falcon included in this study. In particular this study aimed to determine the genetic diversity and population structure of the KwaZulu-Natal Amur falcon. Given the recent increase in hunting of these birds the genetic data was also used to test for the presence of a recent bottleneck in the population.

Genetic diversity in populations is considered a key element to survival (Booy et al, 2000). Genetic diversity allows for a population to adapt to different conditions (Booy et al, 2000), which may be particularly important in migratory species which travel through many different environments. Population genetics theory predicts that a high level of genetic diversity and low inbreeding are indicative of healthy population. The overall observed heterozygosity in the Amur falcon was high (0.67) with low levels of inbreeding (0.14) in comparison to other closely related species such as the migratory Peregrine falcon (*Falco peregrinus*) which showed a mean observed heterozygosity of 0.5 and high inbreeding levels above 0.20 (Ponnikas et al, 2017), as well as the migratory American kestrel (*Falco sparverius*; Ho = 0.50;

Miller et al, 2012). A comparison of genetic diversity estimates from resident birds is needed to determine how migratory gene flowinfluences genetic diversity. The resident American kestrel (*Falco paulus;* Ho = 0.498; Miller et al, 2012), and the resident common kestrel (*Falco tinnunculus;* Ho = 0.22-0.56; Hille et al, 2003) had a lower observed heterozygosity compared to the Amur falcon.

Gene flow, natural selection, and genetic drift are key elements that shape the structure of a population (Eckert et al, 2008; Lesica & Allendorf, 1995; Radosavljević et al, 2015). Very shallow population structure was recovered from the KwaZulu-Natal Amur falcon in both the Bayesian clustering analysis and the PCoA. This suggests that the over wintering two roosts belong to a single large population.. This study contributes towards a growing body of literature suggesting that many bird species have limited population structure due to high dispersal capacity (Nemesházi et al, 2018; Payne, 1991; Stenzel et al, 1994; Weatherhead & Forbes, 1994).However due to the breeding populations being unknown more evidence may be needed to attribute the lack of opulation structure to the dispersal cacity of the species.The lack of population structure may be explained by a lack of migratory connectivity due to random mating within the population. However the shallow population structure could also be a result of different breeding populations within close proximity creating a strong migratory connectivity. Further studies would need to be made in order to confirm if the lack of population structure is due to migratory connectivity or high dispersal capacities.

Despite Amur falcons being targeted by hunting (Bouwman et al, 2012; India, 2012), this study found no evidence for a recent bottleneck. An excess of heterozygotes is a key characteristic seen in a population that has recently seen a bottleneck event (Luikart & Cornuet, 1998). However as time passes there will be an excess of homozygotes due to genetic drift and inbreeding (Luikart & Cornuet, 1998). There was no evidence of heterozygous excess in the current study, which would have been a key signature of a recent bottleneck. However the homozygous excess may indicate that a bottleneck event occurred in the past. The effective population size (Ne) was considerably lower than a census taken in South Africa in 2009. This is expected as the population census included all individuals, not just breeding individuals

The results from this study provide novel information on genetic diversity and population structure of the Amur falcon which has not been investigated previously. It is important to note that although population structure was weak with the populations from over wintering sites, this does not necessarily reflect that the species has weak population structure. Further studies on other population of the Amur falcon could provide a bigger picture of the

population structure within the species. The data in this study can be used as a baseline to access the health of the Amur falcon populations in future.

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Chapter 4: Drivers of *Haemoproteus* parasite infections in the Amur falcon (*Falco amurensis*)

Abstract

Many studies have tried to determine which factors drive haemosporidian infections in bird populations. Key factors such as sex, age and individual heterozygosity have been of particular interest. Heterozygous individuals are expected to be less infected by parasites, with male birds more prone to infections during the breeding season and younger chicks being more susceptible. To test this hypothesis 178 Amur falcons (*Falco amurensis*) were screened for *Haemoproteus* and each individual was genotyped using a suite of microsatellite markers. Mixed effects logistic regression models were run in order to determine if age, sex or individual heterozygosity were significant driving factors in *Haemoproteus* infections. No factors were found to be significant when the genetic diversity present within *Haemoproteus* was not considered. When different lineages of *Haemoproteus* were considered, Lineage A was almost significantly associated with heterogeneity. The regression plots showed an increase in heterozygosity resulted in a greater probability of being infected, this was not an expected result however and may be due to the relationship between heterozygosity and infection rate being quadric and not linear which has been shown in other studies. This study provides baseline information for the driving factors in *Haemoproteus* infections in raptors however studies using different markers that measure heterozygosity in functional genes such as the genes involved in immune response may be more insightful.

4.1 Introduction

There is a growing body of evidence highlighting the synergistic effects of host traits, landscape and climate on parasite transmission in birds at both local and global scales (Fecchio et al, 2021; Padilla et al, 2017; Pérez-Tris & Bensch, 2005; Popescu et al, 2020; Sehgal, 2015). Infection patterns are driven by the interplay between biotic and abiotic conditions (Loiseau et al, 2010). Parasitic infection is dependent on several factors including the hosts' immune system (Calero-Riestra & García, 2016) and the presence of vectors (Balls et al, 2004; van Hoesel et al, 2019). Host immune system status is often linked to age and sex of the host (Calero-Riestra & García, 2016). For example, there is evidence that sex hormones impact the immune system. In particular, testosterone suppresses the immune system making males more vulnerable to infection during the breeding season (Folstad & Karter, 1992; Grossman, 1985; Saino et al, 1995; Schuurs & Verheul, 1990). Specifically, in birds, it has been shown that

immunity is costly and can involve a compromise in psychological or ecological functions during reproduction which ultimately can result in a difference in parasite infection prevalence between the sexes (Sheldon & Verhulst, 1996).Females are less likely to be infected than males (Dawson & Bortolotti, 2001). Factors that are not genetic are also important to consider such as nestlings being highly susceptible to infection as they are confined to the nest and are reliant on adult birds for nutrients to fight infections, as they may also influence the infection rate (Merino, 2010). It is expected that younger birds will have higher infection rates as the immune system is still developing (Rousset et al, 1996).

Genetic diversity is also important for individual fitness as increased genetic diversity has been linked to improved survival and ability to respond to changing environmental conditions (Chapman et al, 2009; Szulkin et al, 2010; Willi et al, 2006) A possible explanation for this is that individuals with more genetic variation are more likely to carry adaptive alleles, particularly alleles that may be involved in immune response and fighting diseases (Keller & Waller, 2002; Reid et al, 2007). Individuals with lower genetic diversity are also more likely to carry deleterious alleles that can have a negative effect on the host and fighting disease (Coltman et al, 1999). The connection between parasitism and genetic diversity can vary across the genome even in regions that are not directly involved in immune response (Szulkin & David, 2011).

Individual heterozygosity can be measured by using a number of different markers, in this case microsatellite markers. Although microsatellites have always been considered neutral markers there is evidence that they may be involved in biological processes (Li et al, 2004). Studies have reported that there is a link between heterozygosity in microsatellite markers and fitness (Hansson & Westerberg, 2002; Mitton & Grant, 1984; Zouros, 1987). There are three hypotheses in the literature that explain this link: The general effect hypothesis, the direct effect hypothesis and the local effect hypothesis. The general effect hypothesis implies that the link between heterozygosity and fitness is genome wide (Cockerham & Weir, 1973). The direct effect implies that the link is affected by a single locus or a few loci (David, 1998) while the local effect implies that disequilibrium linkage between loci results in the link (David, 1998). It is still not well understood how these mechanisms work and how individual genetic diversity impacts host-parasite interactions.

The five most common heterozygosity-fitness correlation measures include the proportion of heterozygosity (PHt; Leary et al, 1984), standardised heterozygosity (SH; Coltman et al, 1999), observed heterozygosity (Ho; Bateson & Saunders, 1902), internal relatedness (IR; Amos et al, 2001) and homozygosity by locus (HL; Aparicio et al, 2006). The

proportion of heterozygosity (PHt), although the simplest method of measuring heterozygosity has been shown to be unreliable when a small number of loci are tested and allele frequencies vary (Aparicio et al, 2006). The standardised heterozygosity (SH) gives the same weight to all markers regardless of varying allele frequencies and it may underestimate the variation at some loci (Aparicio et al, 2006). Internal relatedness (IR) measures parental half genotypes within an individual however it has an asymmetrical distribution due to the allele frequencies not being regarded (Aparicio et al, 2006). Homozygosity by locus (HL) was designed in order to resolve some of the issues with IR estimates and is useful for estimations with a small number of loci as the most informative loci are weighted the most (Aparicio et al, 2006). In migrating species HL is a more useful estimate than IR as immigrants are likely to bring rare alleles and rare alleles are highly overestimated by HL estimates (Aparicio et al, 2006). Each of these heterozygous indices are useful, but some may be more effective than others depending on the dynamics of the populations being studied (Aparicio et al, 2006).

This study aims to determine if sex (male or female), age (juvenile or adult), or individual heterozygosity (measured using five heterozygosity indices estimated from microsatellite date) influences avian *Haemoproteus* infections in the Amur falcon.

4.2 Materials and methods

4.2.1 Data: *Haemoproteus* infections

One hundred and seventy eight Amur falcon samples were used in this study. All samples were collected from two mass hailstorm events which occurred in the KwaZulu-Natal midlands, South Africa (Mooi River and Newcastle) resulting in the mortality of hundreds of falcons (10th and 21st March 2019). Examining birds collected at the same time removes the bias of temporal shifts in infection.

All samples were stored at the Durban Natural Science Museum where birds were sampled. Age (juvenile\ adult) and sex (male\female) were recorded based on appearance. Males have a slate-blue back and a solid breast while females have a spotted reddish brown underside. Juvenile Amur falcons have paler heads and necks as well as black streaks under the wings. *Haemoproteus* infection status was determined using a nested PCR method. The cytochrome *b* gene was amplified from *Haemoproteus* in the blood of infected birds. The first round of amplification used primers HaemNFI/HaemNR3 (Hellgren et al, 2004b) and these PCR products were used as template in the second round of amplification which used HaemF/HaemR2 (Bensch et al, 2000a). Details of the PCR and sequencing conditions are

available in Chapter 2. *Haemoproteus* sequences were then aligned and phylogenetic analyses were conducted (for details see Chapter 2). Phylogenetic analyses showed two lineages of *Haemoproteus* that infect the Amur falcon, Lineage A which infected 81% of infected falcons and Lineage B which infected the remaining 19% of the Amur falcons.

4.2.2 Data: Individual heterozygosity

The individual heterozygosity of each host Amur falcon was estimated using eight microsatellite loci (see Chapter 3 for details). Individual heterozygosity was measured in five different ways, namely proportion of heterozygous loci (PHt; Leary et al, 1984), standardized heterozygosity (Hs; Coltman et al. 1999), internal relatedness (IR; Amos et al. 2001), observed Heterozygosity (Ho) and homozygosity by locus (HL; Aparicio et al. 2006). The five heterozygosity indices were calculated in GenHET (Supplementary Table 4.1; Coulon, 2010).

4.2.3 Mixed-effects logistic regression models

Mixed-effects logistic regression models were used to determine if age (adult or juvenile), sex (male or female), or individual heterozygosity influenced *Haemoproteus* infection status in the Amur falcon. Positive *Haemoproteus* infection was used as the binary response variable with presence of the parasite being coded as 1 and absence being coded as 0. The locality (Mooi River or Newcastle) of the host was included as a random variable. The models included a mixture of continuous and categorical effects. These were age (juvenile/adult), sex (male/female), and individual heterozygosity. Model analyses were run using R4.1.1 (Team, 2013) and RStudio 1.4.1717 (RStudio, 2016). Mixed-effects logistic regression models with a binomial error structure were used for all model analyses. All models were built using the R package LME4 (Bates et al, 2018). Models were run considering all *Haemoproteus* infections, thereafter genetic diversity within *Haemoproteus* was considered with each lineage (Lineage A and Lineage B) recovered by phylogenetic analyses (Chapter 2) run independently. Each heterozygous index was run as separate models due to the strong correlations between the five different indices. For simplicity and due to similarity in results of the heterozygous measures (SH, Ho and PHt) and homozygous measures (HL and IR) only one of each is shown in the results section. For the results section HL was selected as the Amur falcon is a migratory species and SH was selected due to the small number of loci however all results are given as supplementary information.

4.3 Results

Of the 178 falcons included in this study 34% were infected with *Haemoproteus.* eighty-one percent of infections belong to Lineage A and only 19% belong to Lineage B (see Chapter 2 for details).

4.3.1 Haemoproteus

In all the models run no significant correlation was observed when running the *Haemoproteus* data as a whole. Sex, age and all heterozygous indices showed no significance in infection status. Due to the lack of significance these results are reported in Supplementary Table 4.2, 4.3, 4.4, 4.5 & 4.6 and the data was run at a lineage level.

4.3.2.Standardized heterozygosity in both Lineage A and B

In all models run considering only Lineage A infections, sex and age had no significance however PHt, Ho, and SH all were approaching significance with a p-value of 0.07 (Table 4.1 and Supplementary Table 4.7 & 4.8). Due to the similar results and for simplicity, only the models results run with SH as the continuous factor are shown. The SH approached significance ($p<0.05$) with a p-value of 0.07 (Table 4.1). Sex had a high p-value of 0.72 compared to age which had a value of 0.17 however both are insignificant (Table 4.1).

Table 4.1: Results of a mixed-effects logistic regression model run on Lineage A data with the continuous factor standardized heterozygosity (SH). Significant values are in bold and values approaching significance are indicated with a *.

	Estimate	**Standard error**	**Statistical value (Z)**	**P-value**
Intercept	-2.43	0.86	-2.83	**<0.01**
Age	-0.50	0.36	-1.37	0.17
Sex	0.13	0.35	0.36	0.72
Standardized Heterozygosity	1.69	0.92	1.84	0.07*

In all models run on Lineage B, sex, age and heterozygous indices (PHt, Ho and SH) were not significant (Supplementary Table 4.10,4.11 and Table 4.3). Both sex and age showed a high p-value of 0.56 and 0.50 respectively meaning both factors have no significant influence on infection status (Table 4.2). SH had the lowest p-value of 0.14 however was not significant (Table 4.2).

Table 4.2: Results of a mixed-effects logistic regression model run on Lineage B data with the continuous factor standardized heterozygosity (SH). Significant values are in bold and approaching significance values are indicated with a *.

	Estimate	**Standard error**	**Statistical value (Z)**	**p-value**
Intercept	-1.73	0.77	-2.27	**0.02**

Age	-0.22	0.33	-0.68	0.50
Sex	0.19	0.32	0.58	0.56
Standardized Heterozygosity	1.22	0.83	1.48	0.14

Of the 34% Haemoproteus infection rate, 81% were Lineage A and the remaining 19% were Lineage B. Lineage A showed a higher mean and median standardized heterozygosity compared to lineage B. Lineage A had a much broader distribution compared to Lineage B. Lineage B shows that the median and 25th percentile have overlapped.

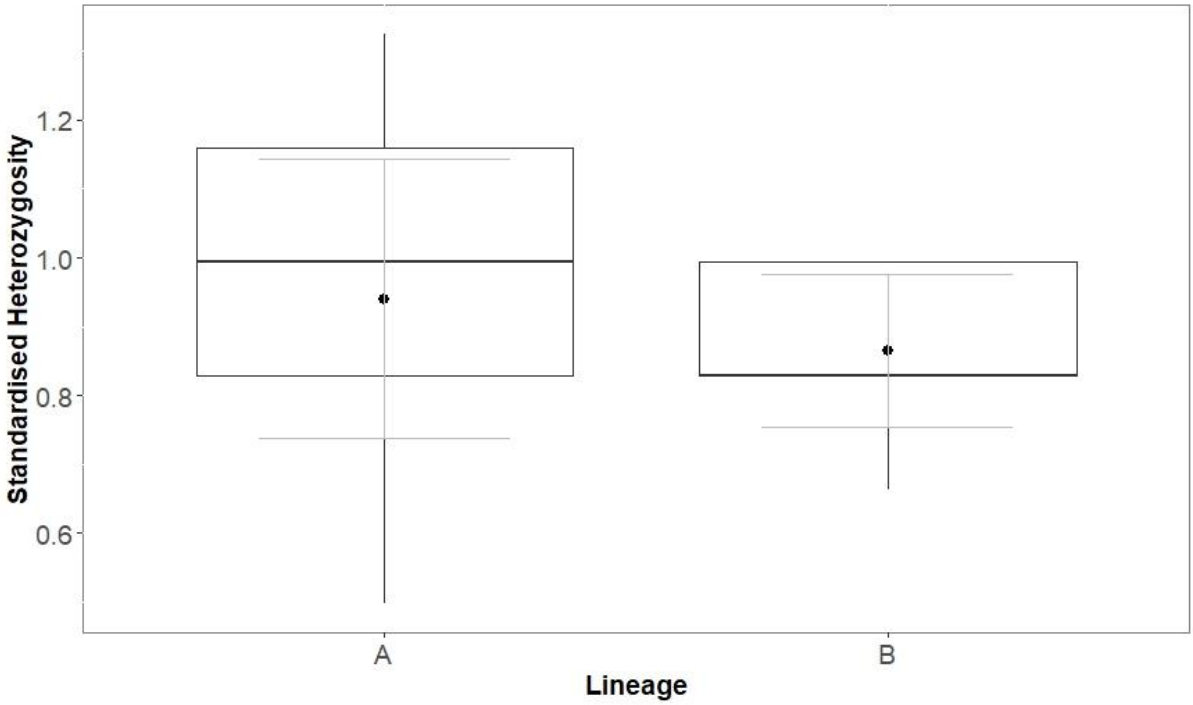

Figure 4.1: Distribution of standardized heterozygosity lineage A and B. Boxplot show the mean, median, interquartile range and a 95% confidence interval.

4.3.3 Homozygous by locus in lineage A and B

Both homozygous measures (IR and HL) showed no significance in sex, age, and homozygosity indices as all p-values were above 0.05 (Supplementary Table 4.9). The results for HL are shown in Table 4.3. Age and HL had similar p-values (0.17 and 0.14) while sex had a high p-value of 0.74 (Table 4.3). However, all variables are statistically insignificant.

Table 4.3: Results of a mixed-effects logistic regression model run on Lineage A data with the continuous factor homozygosity by locus (HL). Significant values are in bold and approaching significance values are indicated with a *.

	Estimate	**Standard error**	**Statistical value (Z)**	**P-value**
Intercept	-0.42	0.46	-0.90	0.37
Age	-0.50	.36	-1.37	0.17
Sex	0.12	0.35	0.34	0.74
Homozygosity by locus	-1.92	1.30	-1.47	0.14

In lineage B Both homozygous measures (IR and HL) showed no significance in sex, age, and homozygosity indices as all p-values were above 0.05 (Table 4.4 and Supplementary Table 4.12). Both HL and age had high p-values of 0.63 and 0.79 respectively (Table 4.4). Sex showed a lower p-value of 0.25 (Table 4.4). However, none of the factors were significant therefore these factors do not influence infection status.

Table 4.4: Results of a mixed-effects logistic regression model run on Lineage bdata with the continuous factor homozygosity by locus (HL).

	Estimate	**Standard error**	**Statistical value (Z)**	**P-value**
Intercept	-3.80	1.03	-3.80	**<0.01**
Age	0.19	0.70	0.27	0.79
Sex	0.85	0.74	1.15	0.25
Homozygosity by locus	1.14	2.38	0.48	0.63

As previously mentioned, of the 34% Haemoproteus infection rate, 81% were Lineage A and the remaining 19% were Lineage B The distribution of homozygosity by locus in lineage B shows a higher mean and median than Lineage A.

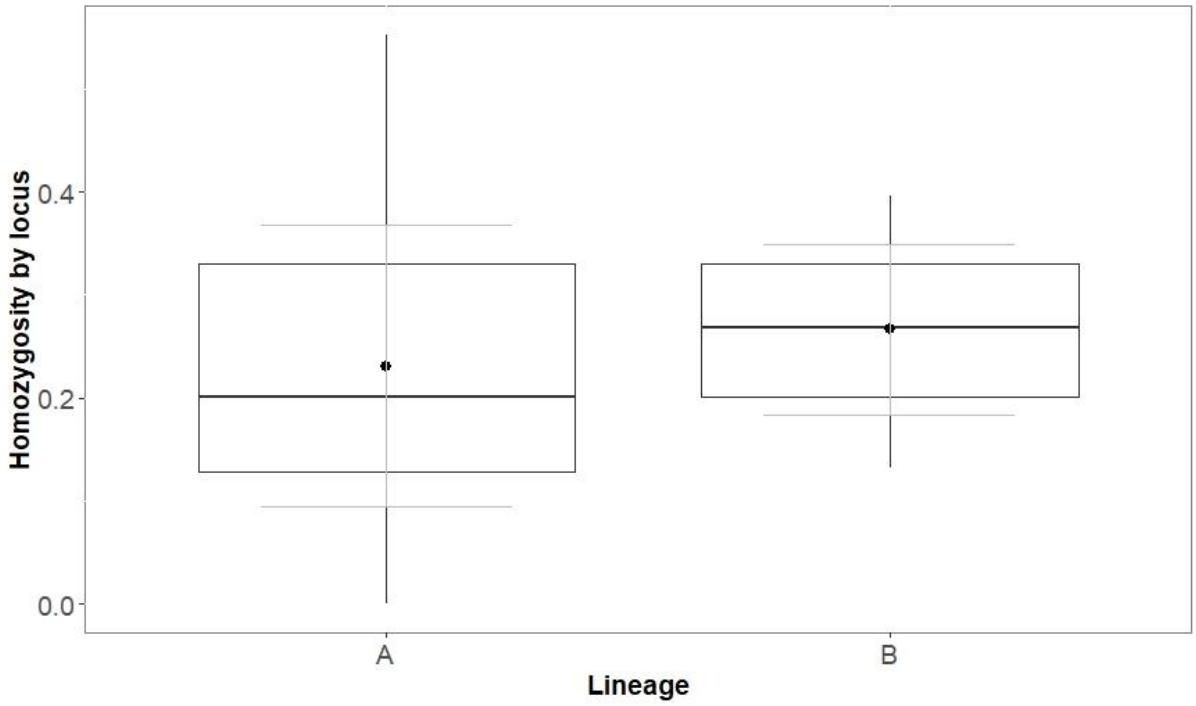

Figure 4.3: Distribution of Homozygosity by locus lineage A and B. Boxplot show the mean, median, interquartile range and a 95% confidence interval.

4.4 Discussion

Many studies have tried to determine which factors influence the infection rate of haemosporidian parasites (Asghar et al, 2015; Dunn et al, 2011; Marzal et al, 2008; Mendes et al, 2005). Factors such as age, sex, and genetic diversity have been of interest when looking at parasite infections (Ferrer et al, 2014; Isaksson et al, 2013). Adult birds have been the focus of most research in haemosporidian parasites however, due to nestlings underdeveloped immune systems, it is assumed that they are more vulnerable to infection (Merino, 2010b) Age showed no statistical significance in any of the models indicating that age does not influence infection status in this study. This result was seen in other studies (Ellis et al, 2014; Illera et al, 2008; Marzal et al, 2008; Ortego et al, 2007). Previous studies have provided evidence indicating that males are expected to have a higher infection rate due to testosterone suppressing the immune system (Hillgarth et al, 1997) however, sex showed no statistical significance in any of the models indicating that sex does not influence infection status in this species. This result has

been confirmed in songbirds and lesser kestrels (Granthon & Williams, 2017; Ortego et al, 2007). This result may change if the birds are screened during the breeding season.

It is well documented that highly homozygous populations are more vulnerable to infections (Charlesworth & Charlesworth, 1987). It would be assumed that more homozygous individuals would be more likely to be infected compared to heterozygous individuals. No significance was seen when looking at the overall *Haemoproteus* infections however when running models at a lineage level a difference was observed in heterozygosity indices and their p-values. Although none of the results showed significance ($p < 0.05$), the models run on Lineage A showed p-values approaching significance ($p = 0.07$) in three heterozygous indices (proportion of loci, standardized heterozygosity and observed heterozygosity). This may indicate that the immune response is linked to individual genetic diversity when the host is infected with particular lineages, in this case, Lineage A had infected 83 percent of the Amur falcons in this study.

It is expected that an individual with a higher heterozygosity will have less chance of infection. . However, a contradictory result occurred in a study that showed a decline in infection status as homozygosity increased (Ferrer et al, 2014). This study showed that individuals that had an HL between 0 and 0.15 showed a decline in infection rate while highly homozygous individuals between 0.15 and 0.40 showed a plateau before infection rate finally declined with individuals that were highly homozygous with an HL above 0.4 (Ferrer et al, 2014). This may explain why infections are occurring in both lineages in hosts with a large variation of Homozygosity as well as standardized heterozygosity. This may also explain why heterozygosity was not significant in the linear regressions. Infection status and heterozygosity may be more of a quadratic relationship and not a linear relationship. This may mean that only individuals with very high SH values will show a decline in infection probability. This indicates that different parasite lineages affect individuals differently.

In conclusion, none of the associations we predicted in this study were significant. Studies have found similar results of no significance when using heterozygosity loci based on microsatellite data (Boerner et al, 2013; Litzke et al, 2019; Ortego et al, 2007). Microsatellite markers may be biased due to the selection of the most polymorphic loci being used in population studies which may make the genome-wide genetic diversity measures inaccurate (Väli et al, 2008). It has also been reported that large panels of microsatellite markers are needed in order to get an accurate measure of HFC's (Boerner et al, 2013; Santure et al, 2010). Further studies using gene families more closely linked to immune response such as the Major Histocompatibility Complex (MHC) or toll-like may hold more information in terms of

individual genetic diversity (Barreiro et al, 2009; Grueber et al, 2012; Spurgin & Richardson, 2010).

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Chapter 5: Conclusions and recommendations for future studies

Haemosporidian parasites may harm the health of their bird hosts (Dawson & Bortolotti, 2000; Merino et al, 2000; Remple, 1981; Remple, 2004b). Raptors are often keystone species in their ecosystems and migrants in particular may introduce novel parasites to local populations (Torchin et al, 2003). This study aimed to determine the prevalence and genetic diversity of haemosporidian parasites in the Amur falcon as well as to examine the genetic diversity and population structure of the Amur falcon. It also aimed to investigate the biotic factors (sex, age and genetic diversity of host) driving haemosporidian infections in the Amur falcon. To date this is the first study to examine genetic diversity in the Amur falcon and haemosporidian parasites infection in the Amur falcon. This study provides valuable information on host-parasite interactions of haemosporidian parasites in the Amur falcon and examines the link between the age, sex and genetic diversity of the host and avian malaria infection.

5.1 Prevalence and host specificity of haemosporidian parasites in the Amur falcon

The Amur falcon is an intercontinental migratory raptor with a broad habitat niche and communal roosting sites which make it a good model species to study host-parasite interactions (Meyburg et al, 2017). Chapter 2 involved screening 178 recently killed Amur falcons for three genera of avian malaria. This unique sample set provides a snapshot of parasite infection in the species. My study found elevated levels of avian malaria infection with 34% of birds screened infected. In particular parasites belonging to the genus *Haemoproteus* were found to be the most prevalent with 60 individuals infected by species belonging to this genus. Parasites belonging to the genus *Leucocytozoon* infected 5 individuals. Parasites belonging to the genus *Plasmodium* were the least prevalent in the sample set, with only a single infection recorded. In future studies, larger sample sizes may produce more *Plasmodium* infections which are key for haemosporidian parasite research as *Plasmodium* is the most detrimental to the host's health (Clark et al, 2015). Another important result for this study is the low level of co-infection (the same individual infected by parasites belonging to two or more genera) I recorded only 2 co-infections in the Amur falcon. By including other parasites isolated from South African raptors and parasite sequences downloaded from GenBank I was also able to test host specificity of the haemosporidian parasites. In Chapter 2 I provide evidence for unique lineages of *Haemoproteus* infecting only the Amur falcon.

5.2 The genetic diversity and population structure of the Amur falcon in KwaZulu-Natal, South Africa

In evolutionary theory, it is assumed that a lack of genetic diversity and high levels of inbreeding can decrease survival and fitness of populations (Charlesworth & Charlesworth, 1987; Falconer, 1996; Lynch & Walsh, 1998). In Chapter 3 I test two hypotheses that have been proposed for migrating species, the first is that genetic diversity increases in migrating populations while the second suggests that migrating populations will homogenize leading to decreased heterozygosity. A lack of population structure is expected in migrating birds due to high dispersal capabilities (Payne, 1991; Stenzel et al, 1994; Weatherhead & Forbes, 1994). In Chapter 3 I described the current genetic diversity of two Amur falcon roosts. One hundred and seventy eight birds were genotyped using a panel of 8 microsatellite markers. Analyses showed higher levels of genetic diversity in the KwaZulu-Natal population in comparison to closely related species belonging to the same genus (*Falco peregrinus, Falco sparverius;* Ponnikas et al, 2017; Miller et al, 2012) indicating a healthy population. Population structure analyses recovered very shallow population partitioning, which was not unexpected given the high dispersal capabilities of the species. However these results may be due to other factors such as high or low migratory connectivity. However due to the lack of knowledge about breeding populations, this result cannot be assumed of the entire species, further studies are needed to determine the population structure of the species. The microsatellite markers used were designed to amplify non-coding regions of the genome. In future studies it would be interesting to examine genetic diversity using functional markers such as immune response genes involved in the adaptive immune system as this may provide a stronger measure of adaptive genetic diversity (Accolla et al, 1995; Mukherjee et al, 2019).

5.3 Factors influencing infection rate in the Amur falcon

Many studies have investigated the factors that influence avian malaria infection (Dunn et al, 2011; Lachish et al, 2013; Marzal et al, 2008; Mendes et al, 2005; Otto, 2003). In Chapter 4 binary regression models were used with *Haemoproteus* infections as the response variable and age, sex, and five heterozygosity indices as predictor variables. Age and sex were not significantly linked to parasite infection in this species. This supports the findings of other studies on avian malaria (Ferraguti et al, 2021; Marzal et al, 2013; Marzal et al, 2008). This does seem to be species dependant as other studies (Asghar et al, 2011; Hammers et al, 2016)

have shown a significant relationship between age, sex and malaria infection in some bird species. Similarly no significant correlation was found between *Haemoproteus* infection and individual heterozygosity. Individuals with higher levels of individual heterozygosity are expected to have better immune systems, but I did not find evidence for this. Interestingly when the different genetic lineages of *Haemoproteus* (recovered from the phylogenetic analyses in Chapter 2) were examined independently, three of the heterozygous indices (PHt, Ho and SH) were approaching significance in Lineage A models. Unexpected findings were found when looking at the heterozygosity indices and their correlation to infection rate. It is well documented that homozygous populations have reduced survival (Hansson & Westerberg, 2002), which was not shown in the results as both lineages showedinfection in a large variation of host heterozygosity. This result may be explained by the quadratic relationship between infection and host heterozygosity inferring that there is a threshold of host heterozygosity that must be reached before a decrease in infection is seen. Although I did use a large data set, sample size may have been an issue here. In addition if individual heterozygosity is measured using genes directly linked to immune response (such as toll-like receptors (TLR) or the Major histocompatibility complex (MHC) gene families) the correlation may be stronger. Further studies would focus on markers that are not neutral as these markers do not provide information on evolutionary variation.

5.4 Conclusions

The results from the different chapters in this thesis provides key baseline information on haemosporidian parasites in raptors(Falconiformes, Strigiformes, Accipitriformes). This study contributes towards a growing body of literature examining host-parasite dynamics in birds.

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Supplementary information

Table S2.1: The details of the 178 Amur falcons (*Falco amurensis*) used in this study

Sample ID	Sex	Age (juvenile/adult)	Locality	Coordinates	*Plasmodium*	*Haemoproteus*	*Leucocytozoon*	Phylogeny code	Network Code
MR714	Female	Adult	Mooi River	29°12'31.0"S 30°00'03.8"E	Negative	Negative	Negative		
MR722	Female	Adult	Mooi River	29°12'31.0"S 30°00'03.8"E	Negative	Negative	Negative		
MR715	Female	Adult	Mooi River	29°12'31.0"S 30°00'03.8"E	Negative	Negative	Negative		
MR725	Male	Adult	Mooi River	29°12'31.0"S 30°00'03.8"E	Negative	Positive	Positive	Amur falcon 1	H24 (L) H38 (H)
MR733	Male	Juvenile	Mooi River	29°12'31.0"S 30°00'03.8"E	Negative	Positive	Negative	Amur falcon 2	H35 (H)
MR785	Male	Adult	Mooi River	29°12'31.0"S 30°00'03.8"E	Negative	Negative	Positive	Amur falcon 60	H24 (L)
MR810	Male	Adult	Mooi River	29°12'31.0"S 30°00'03.8"E	Negative	Negative	Negative		
MR740	Female	Juvenile	Mooi River	29°12'31.0"S 30°00'03.8"E	Negative	Negative	Negative		
MR820	Male	Adult	Mooi River	29°12'31.0"S 30°00'03.8"E	Negative	Negative	Negative		
MR822	Female	Adult	Mooi River	29°12'31.0"S 30°00'03.8"E	Negative	Positive	Negative	Amur falcon 3	H41 (H)
MR818	Male	Adult	Mooi River	29°12'31.0"S 30°00'03.8"E	Negative	Negative	Negative		
MR825	Female	Adult	Mooi River	29°12'31.0"S 30°00'03.8"E	Negative	Positive	Negative	Amur falcon 4	H41 (H)
MR821	Female	Adult	Mooi River	29°12'31.0"S 30°00'03.8"E	Negative	Positive	Negative	Amur falcon 5	H27 (H)

Sample ID	Sex	Age (juvenile/adult)	Locality	Coordinates	*Plasmodium*	*Haemoproteus*	*Leucocytozoon*	Phylogeny code	Network Code
MR827	Male	Adult	Mooi River	29°12'31.0"S 30°00'03.8"E	Negative	Negative	Negative		
MR728	Female	Adult	Mooi River	29°12'31.0"S 30°00'03.8"E	Negative	Negative	Negative		
MR826	Female	Juvenile	Mooi River	29°12'31.0"S 30°00'03.8"E	Negative	Negative	Negative		
MR756	Male	Juvenile	Mooi River	29°12'31.0"S 30°00'03.8"E	Negative	Positive	Negative	Amur falcon 6	H35 (H)
MR823	Female	Juvenile	Mooi River	29°12'31.0"S 30°00'03.8"E	Negative	Negative	Negative		
MR753	Male	Juvenile	Mooi River	29°12'31.0"S 30°00'03.8"E	Negative	Positive	Negative	Amur falcon 7	H29 (H)
MR745	Female	Adult	Mooi River	29°12'31.0"S 30°00'03.8"E	Negative	Negative	Negative		
MR824	Male	Juvenile	Mooi River	29°12'31.0"S 30°00'03.8"E	Negative	Positive	Negative	Amur falcon 8	H27 (H)
MR761	Male	Adult	Mooi River	29°12'31.0"S 30°00'03.8"E	Negative	Negative	Negative		
MR819	Male	Adult	Mooi River	29°12'31.0"S 30°00'03.8"E	Negative	Negative	Negative		
MR775	Male	Adult	Mooi River	29°12'31.0"S 30°00'03.8"E	Negative	Positive	Negative	Amur falcon 9	H41 (H)
MR718	Male	Adult	Mooi River	29°12'31.0"S 30°00'03.8"E	Negative	Negative	Negative		
MR754	Female	Adult	Mooi River	29°12'31.0"S 30°00'03.8"E	Negative	Negative	Negative		
MR762	Male	Adult	Mooi River	29°12'31.0"S 30°00'03.8"E	Negative	Positive	Negative	Amur falcon 10	H27 (H)
MR782	Female	Adult	Mooi River	29°12'31.0"S 30°00'03.8"E	Negative	Negative	Negative		
MR782	Female	Adult	Mooi River	29°12'31.0"S 30°00'03.8"E	Negative	Negative	Negative		

Sample ID	Sex	Age (juvenile/adult)	Locality	Coordinates	*Plasmodium*	*Haemoproteus*	*Leucocytozoon*	Phylogeny code	Network Code
MR760	Male	Adult	Mooi River	29°12'31.0"S 30°00'03.8"E	Negative	Positive	Negative	Amur falcon 11	H27 (H)
MR776	Female	Adult	Mooi River	29°12'31.0"S 30°00'03.8"E	Negative	Negative	Negative		
MR751	Male	Adult	Mooi River	29°12'31.0"S 30°00'03.8"E	Negative	Negative	Negative		
MR774	Male	Adult	Mooi River	29°12'31.0"S 30°00'03.8"E	Negative	Negative	Negative		
MR746	Male	Adult	Mooi River	29°12'31.0"S 30°00'03.8"E	Negative	Negative	Negative		
MR765	Female	Juvenile	Mooi River	29°12'31.0"S 30°00'03.8"E	Negative	Negative	Negative		
MR783	Female	Juvenile	Mooi River	29°12'31.0"S 30°00'03.8"E	Negative	Positive	Negative	Amur falcon 12	H27 (H)
MR768	Male	Adult	Mooi River	29°12'31.0"S 30°00'03.8"E	Negative	Positive	Negative	Amur falcon 13	H27 (H)
MR752	Male	Adult	Mooi River	29°12'31.0"S 30°00'03.8"E	Negative	Positive	Negative	Amur falcon 14	H26 (H)
MR748	Female	Juvenile	Mooi River	29°12'31.0"S 30°00'03.8"E	Negative	Positive	Negative	Amur falcon 15	H27 (H)
MR757	Female	Juvenile	Mooi River	29°12'31.0"S 30°00'03.8"E	Negative	Positive	Positive	Amur falcon 16	H24 (L)
MR755	Female	Juvenile	Mooi River	29°12'31.0"S 30°00'03.8"E	Negative	Negative	Negative		
MR779	Female	Adult	Mooi River	29°12'31.0"S 30°00'03.8"E	Negative	Positive	Negative	Amur falcon 17	H30 (H)
MR747	Female	Adult	Mooi River	29°12'31.0"S 30°00'03.8"E	Negative	Negative	Negative		
MR780	Female	Adult	Mooi River	29°12'31.0"S 30°00'03.8"E	Negative	Positive	Negative	Amur falcon 18	H27 (H)
MR782	Female	Adult	Mooi River	29°12'31.0"S 30°00'03.8"E	Negative	Negative	Negative	MR782	Female

Sample ID	Sex	Age (juvenile/adult)	Locality	Coordinates	*Plasmodium*	*Haemoproteus*	*Leucocytozoon*	Phylogeny code	Network Code
MR755	Female	Juvenile	Mooi River	29°12'31.0"S 30°00'03.8"E	Negative	Negative	Negative		
MR807	Female	Adult	Mooi River	29°12'31.0"S 30°00'03.8"E	Negative	Negative	Negative		
MR769	Male	Juvenile	Mooi River	29°12'31.0"S 30°00'03.8"E	Negative	Negative	Negative		
MR772	Female	Adult	Mooi River	29°12'31.0"S 30°00'03.8"E	Negative	Positive	Negative	Amur falcon 19	H27 (H)
MR750	Female	Adult	Mooi River	29°12'31.0"S 30°00'03.8"E	Negative	Negative	Negative		
MR764	Female	Adult	Mooi River	29°12'31.0"S 30°00'03.8"E	Negative	Negative	Negative		
MR759	Female	Adult	Mooi River	29°12'31.0"S 30°00'03.8"E	Negative	Negative	Negative		
N3	Male	Adult	Newcastle	29°12'31.0"S 30°00'03.8"E	Negative	Negative	Negative		
N6	Male	Juvenile	Newcastle	29°12'31.0"S 30°00'03.8"E	Negative	Negative	Negative		
N9	Male	Adult	Newcastle	29°12'31.0"S 30°00'03.8"E	Negative	Positive	Negative	Amur falcon 25	H35 (H)
N10	Male	Adult	Newcastle	29°12'31.0"S 30°00'03.8"E	Negative	Negative	Negative		
N23	Male	Adult	Newcastle	29°12'31.0"S 30°00'03.8"E	Negative	Negative	Negative		
N26	Male	Adult	Newcastle	29°12'31.0"S 30°00'03.8"E	Negative	Negative	Negative		
N33	Male	Adult	Newcastle	29°12'31.0"S 30°00'03.8"E	Negative	Positive	Negative	Amur falcon 26	H34 (H)
N48	Male	Adult	Newcastle	29°12'31.0"S 30°00'03.8"E	Negative	Positive	Negative	Amur falcon 32	H27 (H)
N134	Male	Juvenile	Newcastle	29°12'31.0"S 30°00'03.8"E	Negative	Negative	Negative		

Sample ID	**Sex**	**Age (juvenile/adult)**	**Locality**	29°12'31.0"S 30°00'03.8"E	***Plasmodium***	***Haemoproteus***	***Leucocytozoon***	**Phylogeny code**	**Network Code**
N136	Male	Juvenile	Newcastle	29°12'31.0"S 30°00'03.8"E	Negative	Negative	Negative		
N211	Male	Juvenile	Newcastle	29°12'31.0"S 30°00'03.8"E	Negative	Positive	Negative	Amur falcon 33	H27 (H)
N214	Female	Adult	Newcastle	29°12'31.0"S 30°00'03.8"E	Negative	Positive	Negative	Amur falcon 21	H32 (H)
N224	Male	Juvenile	Newcastle	29°12'31.0"S 30°00'03.8"E	Negative	Negative	Negative		
N233	Male	Juvenile	Newcastle	29°12'31.0"S 30°00'03.8"E	Negative	Negative	Negative		
N257	Male	Adult	Newcastle	29°12'31.0"S 30°00'03.8"E	Negative	Negative	Negative		
N254	Male	Juvenile	Newcastle	29°12'31.0"S 30°00'03.8"E	Negative	Negative	Negative		
N305	Female	Adult	Newcastle	29°12'31.0"S 30°00'03.8"E	Negative	Negative	Negative		
N308	Female	Juvenile	Newcastle	29°12'31.0"S 30°00'03.8"E	Negative	Positive	Negative	Amur falcon 35	H27 (H)
N317	Female	Juvenile	Newcastle	29°12'31.0"S 30°00'03.8"E	Negative	Positive	Negative	Amur falcon 24	H27 (H)
N352	Female	Adult	Newcastle	29°12'31.0"S 30°00'03.8"E	Negative	Positive	Negative	Amur falcon 22	H27 (H)
N355	Female	Juvenile	Newcastle	29°12'31.0"S 30°00'03.8"E	Negative	Negative	Negative		
N356	Female	Adult	Newcastle	29°12'31.0"S 30°00'03.8"E	Negative	Negative	Negative		
N461	Male	Adult	Newcastle	29°12'31.0"S 30°00'03.8"E	Negative	Negative	Negative		
N468	Male	Adult	Newcastle	29°12'31.0"S 30°00'03.8"E	Negative	Positive	Negative	Amur falcon 20	H27 (H)
N484	Male	Adult	Newcastle	29°12'31.0"S 30°00'03.8"E	Negative	Negative	Negative		

Sample ID	**Sex**	**Age (juvenile/adult)**	**Locality**	29°12'31.0"S 30°00'03.8"E	***Plasmodium***	***Haemoproteus***	***Leucocytozoon***	**Phylogeny code**	**Network Code**
N486	Male	Adult	Newcastle	29°12'31.0"S 30°00'03.8"E	Negative	Positive	Negative	Amur falcon 28	H28 (H)
N490	Male	Adult	Newcastle	29°12'31.0"S 30°00'03.8"E	Negative	Negative	Negative		
N494	Male	Adult	Newcastle	29°12'31.0"S 30°00'03.8"E	Negative	Positive	Negative	Amur falcon 31	H27 (H)
N499	Female	Adult	Newcastle	29°12'31.0"S 30°00'03.8"E	Negative	Negative	Negative		
N551	Male	Juvenile	Newcastle	29°12'31.0"S 30°00'03.8"E	Negative	Positive	Negative	Amur falcon 36	H31(H)
N553	Male	Juvenile	Newcastle	29°12'31.0"S 30°00'03.8"E	Negative	Negative	Negative		
N575	Female	Adult	Newcastle	29°12'31.0"S 30°00'03.8"E	Negative	Positive	Negative	Amur falcon 23	H37 (H)
N590	Female	Juvenile	Newcastle	29°12'31.0"S 30°00'03.8"E	Negative	Positive	Negative	Amur falcon 37	H27 (H)
N890	Female	Adult	Newcastle	29°12'31.0"S 30°00'03.8"E	Negative	Positive	Negative		
N594	Female	Juvenile	Newcastle	29°12'31.0"S 30°00'03.8"E	Negative	Positive*	Negative		
N597	Male	Adult	Newcastle	29°12'31.0"S 30°00'03.8"E	Negative	Negative	Negative		
N599	Female	Adult	Newcastle	29°12'31.0"S 30°00'03.8"E	Negative	Negative	Negative		
N602	Female	Adult	Newcastle	29°12'31.0"S 30°00'03.8"E	Negative	Positive	Negative	Amur falcon 27	H41 (H)
N605	Female	Adult	Newcastle	29°12'31.0"S 30°00'03.8"E	Negative	Negative	Negative		
N610	Female	Adult	Newcastle	29°12'31.0"S 30°00'03.8"E	Negative	Negative	Negative		
N857	Female	Juvenile	Newcastle	29°12'31.0"S 30°00'03.8"E	Negative	Positive	Negative	Amur falcon 30	H29 (H)

Sample ID	**Sex**	**Age (juvenile/adult)**	**Locality**	29°12'31.0"S 30°00'03.8"E	***Plasmodium***	***Haemoproteus***	***Leucocytozoon***	**Phylogeny code**	**Network Code**
N885	Female	Juvenile	Newcastle	29°12'31.0"S 30°00'03.8"E	Negative	Negative	Negative		
N886	Female	Juvenile	Newcastle	29°12'31.0"S 30°00'03.8"E	Negative	Negative	Negative		
N894	Female	Juvenile	Newcastle	29°12'31.0"S 30°00'03.8"E	Negative	Negative	Negative		
N904	Female	Adult	Newcastle	29°12'31.0"S 30°00'03.8"E	Negative	Negative	Negative		
N913	Female	Juvenile	Newcastle	29°12'31.0"S 30°00'03.8"E	Negative	Positive	Negative	Amur falcon 29	H33 (H)
N925	Female	Adult	Newcastle	29°12'31.0"S 30°00'03.8"E	Negative	Negative	Negative		
N1149	Male	Juvenile	Newcastle	29°12'31.0"S 30°00'03.8"E	Negative	Positive	Negative	Amur falcon 34	H36 (H)
711	Male	Adult	Newcastle	29°12'31.0"S 30°00'03.8"E	Negative	Negative	Negative		
168	Male	Adult	Newcastle	29°12'31.0"S 30°00'03.8"E	Negative	Positive	Negative	Amur falcon 38	H27 (H)
125	Male	Adult	Newcastle	29°12'31.0"S 30°00'03.8"E	Negative	Negative	Negative		
665	Male	Adult	Newcastle	29°12'31.0"S 30°00'03.8"E	Negative	Positive	Negative	Amur falcon 39	H27 (H)
N315	Female	Adult	Newcastle	29°12'31.0"S 30°00'03.8"E	Negative	Negative	Negative		
N628	Female	Adult	Newcastle	29°12'31.0"S 30°00'03.8"E	Negative	Negative	Negative		
N390	Female	Adult	Newcastle	29°12'31.0"S 30°00'03.8"E	Negative	Negative	Negative		
N393	Female	Adult	Newcastle	29°12'31.0"S 30°00'03.8"E	Negative	Positive	Negative	Amur falcon 40	H27 (H)
N324	Female	Adult	Newcastle	29°12'31.0"S 30°00'03.8"E	Negative	Negative	Negative		

Sample ID	**Sex**	**Age (juvenile/adult)**	**Locality**	29°12'31.0"S 30°00'03.8"E	***Plasmodium***	***Haemoproteus***	***Leucocytozoon***	**Phylogeny code**	**Network Code**
N354	Female	Adult	Newcastle	29°12'31.0"S 30°00'03.8"E	Negative	Negative	Negative		
N335	Female	Adult	Newcastle	29°12'31.0"S 30°00'03.8"E	Negative	Negative	Negative		
N569	Female	Adult	Newcastle	29°12'31.0"S 30°00'03.8"E	Negative	Negative	Negative		
157	Male	Adult	Newcastle	29°12'31.0"S 30°00'03.8"E	Negative	Positive	Negative	Amur falcon 41	H27 (H)
N212	Male	Adult	Newcastle	29°12'31.0"S 30°00'03.8"E	Negative	Positive	Negative	Amur falcon 42	H27 (H)
N210	Male	Adult	Newcastle	29°12'31.0"S 30°00'03.8"E	Negative	Positive	Negative	Amur falcon 43	H27 (H)
N230	Male	Adult	Newcastle	29°12'31.0"S 30°00'03.8"E	Negative	Negative	Negative		
N203	Male	Adult	Newcastle	29°12'31.0"S 30°00'03.8"E	Negative	Positive	Negative	Amur falcon 44	H27 (H)
N219	Male	Adult	Newcastle	29°12'31.0"S 30°00'03.8"E	Negative	Negative	Negative		
N239	Male	Adult	Newcastle	29°12'31.0"S 30°00'03.8"E	Negative	Positive	Negative	Amur falcon 45	H27 (H)
N614	Female	Adult	Newcastle	29°12'31.0"S 30°00'03.8"E	Negative	Negative	Negative		
N860	Female	Adult	Newcastle	29°12'31.0"S 30°00'03.8"E	Negative	Negative	Negative		
663	Male	Adult	Newcastle	29°12'31.0"S 30°00'03.8"E	Negative	Negative	Negative		
607	Male	Adult	Newcastle	29°12'31.0"S 30°00'03.8"E	Negative	Negative	Negative		
167	Male	Adult	Newcastle	29°12'31.0"S 30°00'03.8"E	Negative	Negative	Negative		
631	Female	Adult	Newcastle	29°12'31.0"S 30°00'03.8"E	Negative	Negative	Negative		

Sample ID	Sex	Age (juvenile/adult)	Locality	Coordinates	*Plasmodium*	*Haemoproteus*	*Leucocytozoon*	Phylogeny code	Network Code
636	Female	Adult	Newcastle	29°12'31.0"S 30°00'03.8"E	Negative	Negative	Negative		
684	Female	Adult	Newcastle	29°12'31.0"S 30°00'03.8"E	Negative	Negative	Negative		
703	Female	Adult	Newcastle	29°12'31.0"S 30°00'03.8"E	Negative	Negative	Negative		
6	Female	Adult	Newcastle	29°12'31.0"S 30°00'03.8"E	Negative	Negative	Negative		
592	Male	Juvenile	Newcastle	29°12'31.0"S 30°00'03.8"E	Negative	Negative	Negative		
N347	Female	Juvenile	Newcastle	-27.754567, 29.985359	Negative	Negative	Negative		
N320	Female	Juvenile	Newcastle	-27.754567, 29.985359	Negative	Negative	Negative		
N365	Female	Juvenile	Newcastle	-27.754567, 29.985359	Negative	Negative	Negative		
N911	Female	Juvenile	Newcastle	-27.754567, 29.985359	Negative	Negative	Negative		
N896	Female	Juvenile	Newcastle	-27.754567, 29.985359	Negative	Negative	Negative		
N377	Female	Juvenile	Newcastle	-27.754567, 29.985359	Negative	Negative	Negative		
677	Male	Juvenile	Newcastle	29°12'31.0"S 30°00'03.8"E	Negative	Negative	Negative		
666	Male	Juvenile	Newcastle	29°12'31.0"S 30°00'03.8"E	Negative	Positive	Negative	Amur falcon 46	H41 (H)
593	Male	Juvenile	Newcastle	29°12'31.0"S 30°00'03.8"E	Negative	Negative	Negative		
105	Male	Juvenile	Newcastle	29°12'31.0"S 30°00'03.8"E	Negative	Negative	Negative		
186	Male	Juvenile	Newcastle	29°12'31.0"S 30°00'03.8"E	Negative	Negative	Negative		

Sample ID	Sex	Age (juvenile/adult)	Locality	Coordinates	*Plasmodium*	*Haemoproteus*	*Leucocytozoon*	Phylogeny code	Network Code
199	Male	Juvenile	Newcastle	29°12'31.0"S 30°00'03.8"E	Negative	Negative	Negative		
71	Male	Juvenile	Newcastle	29°12'31.0"S 30°00'03.8"E	Negative	Negative	Negative		
127	Male	Juvenile	Newcastle	29°12'31.0"S 30°00'03.8"E	Negative	Negative	Negative		
111	Male	Juvenile	Newcastle	29°12'31.0"S 30°00'03.8"E	Negative	Negative	Negative		
589	Female	Adult	Newcastle	29°12'31.0"S 30°00'03.8"E	Negative	Negative	Negative		
334	Female	Adult	Newcastle	29°12'31.0"S 30°00'03.8"E	Negative	Negative	Negative		
691	Female	Adult	Newcastle	29°12'31.0"S 30°00'03.8"E	Negative	Negative	Negative		
N645	Female	Juvenile	Newcastle	29°12'31.0"S 30°00'03.8"E	Negative	Negative	Negative		
538	Female	Adult	Newcastle	29°12'31.0"S 30°00'03.8"E	Negative	Positive	Negative	Amur falcon 47	H27 (H)
646	Female	Adult	Newcastle	29°12'31.0"S 30°00'03.8"E	Negative	Positive#	Negative		
N375	Male	Juvenile	Newcastle	29°12'31.0"S 30°00'03.8"E	Negative	Negative	Negative		
N389	Male	Juvenile	Newcastle	29°12'31.0"S 30°00'03.8"E	Negative	Negative	Negative		
N387	Male	Juvenile	Newcastle	29°12'31.0"S 30°00'03.8"E	Negative	Negative	Negative		
N191	Male	Juvenile	Newcastle	29°12'31.0"S 30°00'03.8"E	Negative	Negative	Negative		
N741	Male	Juvenile	Newcastle	29°12'31.0"S 30°00'03.8"E	Negative	Positive	Negative	Amur falcon 48	H27 (H)
N351	Male	Juvenile	Newcastle	29°12'31.0"S 30°00'03.8"E	Negative	Negative	Negative		

Sample ID	**Sex**	**Age (juvenile/adult)**	**Locality**	29°12'31.0"S 30°00'03.8"E	***Plasmodium***	***Haemoproteus***	***Leucocytozoon***	**Phylogeny code**	**Network Code**
N357	Female	Juvenile	Newcastle	29°12'31.0"S 30°00'03.8"E	Negative	Positive	Negative	Amur falcon 49	H27 (H)
N893	Female	Juvenile	Newcastle	29°12'31.0"S 30°00'03.8"E	Negative	Positive	Negative	Amur falcon 50	H27 (H)
N595	Female	Juvenile	Newcastle	29°12'31.0"S 30°00'03.8"E	Negative	Negative	Negative		
184	Female	Juvenile	Newcastle	29°12'31.0"S 30°00'03.8"E	Negative	Negative	Negative		
602	Female	Juvenile	Newcastle	29°12'31.0"S 30°00'03.8"E	Negative	Negative	Negative		
681	Female	Juvenile	Newcastle	29°12'31.0"S 30°00'03.8"E	Negative	Positive	Negative	Amur falcon 51	H27 (H)
242	Female	Juvenile	Newcastle	29°12'31.0"S 30°00'03.8"E	Negative	Negative	Negative		
188	Female	Juvenile	Newcastle	29°12'31.0"S 30°00'03.8"E	Negative	Positive	Negative	Amur falcon 52	H23 (H)
N231	Male	Juvenile	Newcastle	29°12'31.0"S 30°00'03.8"E	Negative	Positive	Negative	Amur falcon 58	H41 (H)
N1150	Male	Juvenile	Newcastle	29°12'31.0"S 30°00'03.8"E	Negative	Negative	Negative		
N40	Male	Juvenile	Newcastle	29°12'31.0"S 30°00'03.8"E	Negative	Positive	Negative	Amur falcon 53	H41 (H)
N1082	Male	Juvenile	Newcastle	29°12'31.0"S 30°00'03.8"E	Negative	Positive	Positive	Amur falcon 54	H24 (L) H27 (H)
N572	Male	Adult	Newcastle	29°12'31.0"S 30°00'03.8"E	Negative	Positive	Positive	Amur falcon 55	H25 (L) H27 (H)
N205	Male	Adult	Newcastle	29°12'31.0"S 30°00'03.8"E	Negative	Positive	Negative	Amur falcon 56	H41 (H)
N250	Male	Adult	Newcastle	29°12'31.0"S 30°00'03.8"E	Negative	Negative	Negative		
N554	Male	Adult	Newcastle	29°12'31.0"S 30°00'03.8"E	Negative	Positive	Negative	Amur falcon 57	H35 (H)

Sample ID	Sex	Age (juvenile/adult)	Locality	29°12'31.0"S 30°00'03.8"E	*Plasmodium*	*Haemoproteus*	*Leucocytozoon*	Phylogeny code	Network Code
202	Female	Juvenile	Newcastle	29°12'31.0"S 30°00'03.8"E	Negative	Negative	Negative		
140	Female	Juvenile	Newcastle	29°12'31.0"S 30°00'03.8"E	Negative	Negative	Negative		
144	Female	Juvenile	Newcastle	29°12'31.0"S 30°00'03.8"E	Negative	Negative	Negative		
91	Female	Juvenile	Newcastle	29°12'31.0"S 30°00'03.8"E	Negative	Positive*	Negative		
175	Female	Juvenile	Newcastle	29°12'31.0"S 30°00'03.8"E	Negative	Negative	Negative		
42	Male	Adult	Newcastle	29°12'31.0"S 30°00'03.8"E	Negative	Negative	Negative		
117	Male	Adult	Newcastle	29°12'31.0"S 30°00'03.8"E	Negative	Negative	Negative		

Table S2.2: The other raptors used in this study

Bird Species	Common Name	Location	Sample Code	Data Code	Plasmodium	Haemoproteus	Leucocytozoon
Buteo rufofuscus	jackal Buzzards	African Bird of Prey Sanctuary	160	QH14	Negative	Negative	Negative
Buteo rufofuscus	jackal Buzzards	North of Himeville	27	QH4	Negative	Negative	Negative
Buteo rufofuscus	jackal Buzzards	Himeville	35	QH12	Negative	Negative	Negative

Bird Species	Common Name	Location	Sample Code	Data Code	Plasmodium	Haemoproteus	Leucocytozoon
Buteo rufofuscus	jackal Buzzards	Upper Backs Valley, Port Elizabeth	110	QH1	Negative	Negative	Negative
Buteo rufofuscus	jackal Buzzards	Unknown	184	QH2	Negative	Negative	Negative
Buteo rufofuscus	jackal Buzzards	Unknown	183	QH3	Negative	Negative	Negative
Buteo rufofuscus	jackal Buzzards	Reins Nature Reserve	180	QH5	Negative	Negative	Negative
Buteo rufofuscus	jackal Buzzards	Wilderness Lakes	175	QH6	Negative	Negative	Negative
Buteo rufofuscus	jackal Buzzards	Unknown	186	QH7	Negative	Negative	Negative
Buteo rufofuscus	jackal Buzzards	Unknown	185	QH8	Negative	Negative	Negative
Buteo rufofuscus	jackal Buzzards	Durban International Airport	118	QH9	Negative	Negative	Negative
Buteo rufofuscus	jackal Buzzards	Hlatimba	9	QH10	Negative	Negative	Negative
Buteo rufofuscus	jackal Buzzards	Highmoor	46	QH11	Negative	Negative	Negative
Buteo rufofuscus	jackal Buzzards	Nottingham Road	60	QH13	Negative	Negative	Negative
Buteo rufofuscus	jackal Buzzards	SW of Nottingham Road	7	QH15	Negative	Negative	Negative
Buteo rufofuscus	jackal Buzzards	Kamberg	19	QH16	Negative	Negative	Negative
Buteo rufofuscus	jackal Buzzards	Glengarry	6	QH17	Negative	Negative	Negative
Buteo rufofuscus	jackal Buzzards	HellaHella	59	QH18	Negative	Negative	Negative

Bird Species	Common Name	Location	Sample Code	Data Code	Plasmodium	Haemoproteus	Leucocytozoon
Buteo rufofuscus	jackal Buzzards	African Bird of Prey Centre	42	QH19	Negative	Negative	Negative
Buteo rufofuscus	jackal Buzzards	P19 Road	13	QH20	Negative	Negative	Negative
Buteo rufofuscus	jackal Buzzards	Drak Gardens Road	21	QH21	Negative	Negative	Negative
Buteo rufofuscus	jackal Buzzards	R626 Road, Underberg	22	QH22	Negative	Negative	Negative
Buteo rufofuscus	jackal Buzzards	Himeville	2	QH23	Negative	Negative	Negative
Buteo rufofuscus	jackal Buzzards	Fort Nottingham	30	QH24	Negative	Negative	Negative
Buteo rufofuscus	jackal Buzzards	Giants Castle Road	34	QH25	Negative	Negative	Negative
Buteo rufofuscus	jackal Buzzards	Coleford Road	28	QH26	Negative	Negative	Negative
Buteo rufofuscus	jackal Buzzards	Nottingham Road	29	QH27	Negative	Negative	Negative
Buteo rufofuscus	jackal Buzzards	Glengarry, Kamberg	32	QH28	Negative	Negative	Negative
Buteo rufofuscus	jackal Buzzards	Giants Castle Road	33	QH29	Negative	Negative	Negative
Buteo rufofuscus	jackal Buzzards	Richmond area	36	QH30	Negative	Negative	Negative
Buteo rufofuscus	jackal Buzzards	Bushman's Nek Road	48	QH31	Negative	Negative	Negative
Buteo rufofuscus	jackal Buzzards	African Bird of Prey Centre	52	QH32	Negative	Negative	Negative
Buteo rufofuscus	jackal Buzzards	Unknown	120	QH33	Negative	Negative	Negative
Buteo rufofuscus	jackal Buzzards	Bushman's Nek Road	24	QH34	Negative	Negative	Negative

Bird Species	Common Name	Location	Sample Code	Data Code	Plasmodium	Haemoproteus	Leucocytozoon
Buteo rufofuscus	jackal Buzzards	Kamberg	25	QH35	Negative	Negative	Negative
Buteo rufofuscus	jackal Buzzards	SW of Nottingham Road	26	QH36	Negative	Negative	Negative
Buteo rufofuscus	jackal Buzzards	Coleford Road	23	QH37	Negative	Negative	Negative
Buteo rufofuscus	jackal Buzzards	P19 Road	13	QH38	Negative	Negative	Negative
Buteo rufofuscus	jackal Buzzards	Karoo Palmiet Drift	168	QH39	Negative	Negative	Negative
Buteo rufofuscus	jackal Buzzards	African Bird of Prey Sanctuary	157	QH40	Negative	Negative	Negative
Buteo rufofuscus	jackal Buzzards	Pietermaritzburg	103	QH41	Negative	Negative	Negative
Buteo rufofuscus	jackal Buzzards	Nordlingen, Bavaria	128	QH42	Negative	Negative	Negative
Buteo rufofuscus	jackal Buzzards	Howick	140	QH43	Negative	Negative	Negative
Buteo rufofuscus	jackal Buzzards	Cedarville flats	68	QH44	Negative	Negative	Negative
Buteo rufofuscus	jackal Buzzards	Ottos Bluff Road	72	QH45	Negative	Negative	Negative
Buteo rufofuscus	jackal Buzzards	Karkloof	73	QH46	Negative	Negative	Negative
Buteo rufofuscus	jackal Buzzards	Mmathis Basutoland	101	QH47	Negative	Negative	Negative
Buteo rufofuscus	jackal Buzzards	Sagana River	106	QH48	Negative	Negative	Negative
Buteo rufofuscus	jackal Buzzards	African Bird of Prey Sanctuary	157	QH49	Negative	Negative	Negative
Gyps africanus	white-backed vultures	Unknown	H066	H1	Negative	Negative	Negative

Bird Species	Common Name	Location	Sample Code	Data Code	Plasmodium	Haemoproteus	Leucocytozoon
Gyps africanus	white-backed vultures	Unknown	H067	H2	Negative	Negative	Negative
Gyps africanus	white-backed vultures	Unknown	H067'	H3	Negative	Negative	Negative
Gyps africanus	white-backed vultures	Unknown	H068	H4	Negative	Negative	Negative
Gyps africanus	white-backed vultures	Unknown	H069	H5	Negative	Negative	Negative
Gyps africanus	white-backed vultures	Unknown	H070	H6	Negative	Negative	Negative
Gyps africanus	white-backed vultures	Unknown	H071	H7	Negative	Negative	Negative
Gyps africanus	white-backed vultures	Unknown	H072	H8	Negative	Negative	Negative
Gyps africanus	white-backed vultures	Unknown	H073	H9	Negative	Negative	Negative
Gyps africanus	white-backed vultures	Unknown	H074	H10	Negative	Negative	Negative
Gyps africanus	white-backed vultures	Unknown	H075	H11	Negative	Negative	Negative
Gyps africanus	white-backed vultures	Unknown	H076	H12	Negative	Negative	Negative
Gyps africanus	white-backed vultures	Unknown	H077	H13	Negative	Negative	Negative
Gyps africanus	white-backed vultures	Unknown	H078	H14	Negative	Negative	Negative
Gyps africanus	white-backed vultures	Unknown	H079	H15	Negative	Negative	Negative
Gyps africanus	white-backed vultures	Unknown	H080	H16	Negative	Negative	Negative
Gyps africanus	white-backed vultures	Unknown	H081	H17	Negative	Negative	Negative

Bird Species	Common Name	Location	Sample Code	Data Code	Plasmodium	Haemoproteus	Leucocytozoon
Gyps africanus	white-backed vultures	Unknown	H082	H18	Negative	Negative	Negative
Gyps africanus	white-backed vultures	Unknown	H083	H19	Negative	Negative	Negative
Gyps africanus	white-backed vultures	Unknown	H084	H20	Negative	Negative	Negative
Gyps africanus	white-backed vultures	Unknown	H085	H21	Negative	Negative	Negative
Gyps africanus	white-backed vultures	African Bird Sancturary	15	DH1	Negative	Negative	Negative
Gyps africanus	white-backed vultures	African Bird Sancturary	16	DH2	Negative	Negative	Negative
Gyps africanus	white-backed vultures	African Bird Sancturary	18	DH3	Negative	Positive	Negative
Gyps africanus	white-backed vultures	African Bird Sancturary	P068 wh	DH4	Negative	Negative	Negative
Gyps africanus	white-backed vultures	African Bird Sancturary	1 or 2	DH5	Negative	Negative	Negative
Gyps africanus	white-backed vultures	African Bird Sancturary	12	DH6	Negative	Negative	Negative
Gyps africanus	white-backed vultures	African Bird Sancturary	11	DH7	Negative	Negative	Negative
Gyps africanus	white-backed vultures	African Bird Sancturary	1	DH8	Negative	Negative	Negative
Gyps africanus	white-backed vultures	African Bird Sancturary	9	DH9	Negative	Negative	Negative
Gyps africanus	white-backed vultures	African Bird Sancturary	7	DH10	Negative	Negative	Negative
Gyps africanus	white-backed vultures	African Bird Sancturary	2	DH11	Negative	Negative	Negative
Gyps africanus	white-backed vultures	African Bird Sancturary	5	DH12	Negative	Negative	Negative

Bird Species	Common Name	Location	Sample Code	Data Code	Plasmodium	Haemoproteus	Leucocytozoon
Gyps africanus	white-backed vultures	African Bird Sancturary	6	DH13	Negative	Negative	Negative
Gyps africanus	white-backed vultures	African Bird Sancturary	8	DH14	Negative	Positive	Negative
Gyps africanus	white-backed vultures	African Bird Sancturary	4	DH15	Negative	Positive	Negative
Gyps africanus	white-backed vultures	African Bird Sancturary	13	DH16	Negative	Negative	Negative
Gyps africanus	white-backed vultures	African Bird Sancturary	3	DH17	Negative	Negative	Negative
Gyps africanus	white-backed vultures	African Bird Sancturary	14	DH18	Negative	Negative	Negative
Gyps africanus	white-backed vultures	African Bird Sancturary	10	DH19	Negative	Negative	Negative
Gyps africanus	white-backed vultures	Unknown	A275	KH1	Negative	Negative	Negative
Gyps africanus	white-backed vultures	Unknown	533724	KH2	Negative	Negative	Negative
Gyps africanus	white-backed vultures	Unknown	P078	KH3	Negative	Negative	Negative
Gyps africanus	white-backed vultures	Unknown	A268	KH4	Negative	Negative	Negative
Gyps africanus	white-backed vultures	Unknown	A272	KH5	Negative	Negative	Negative
Gyps africanus	white-backed vultures	Unknown	533754	KH6	Negative	Negative	Negative
Gyps africanus	white-backed vultures	Unknown	63354	KH7	Negative	Negative	Negative
Gyps africanus	white-backed vultures	Unknown	A262	KH8	Negative	Negative	Negative
Gyps africanus	white-backed vultures	Unknown	5015	KH9	Negative	Negative	Negative

Bird Species	Common Name	Location	Sample Code	Data Code	Plasmodium	Haemoproteus	Leucocytozoon
Gyps africanus	white-backed vultures	Unknown	2008	KH10	Negative	Positive	Negative
Gyps africanus	white-backed vultures	Unknown	633757	KH11	Negative	Negative	Negative
Gyps africanus	white-backed vultures	Unknown	633733	KH12	Negative	Negative	Negative
Gyps africanus	white-backed vultures	Unknown	M047	KH13	Negative	Negative	Negative
Gyps africanus	white-backed vultures	Unknown	533722	KH14	Negative	Negative	Negative
Gyps africanus	white-backed vultures	Unknown	632565	KH15	Negative	Negative	Negative
Gyps africanus	white-backed vultures	Unknown	A252	KH16	Negative	Negative	Negative
Gyps africanus	white-backed vultures	Unknown	528912	KH17	Negative	Negative	Negative
Gyps africanus	white-backed vultures	Unknown	2006	KH18	Negative	Negative	Negative
Gyps africanus	white-backed vultures	Unknown	2005	KH19	Negative	Negative	Negative
Gyps africanus	white-backed vultures	Unknown	532992	KH20	Negative	Negative	Negative
Gyps africanus	white-backed vultures	Unknown	A274	KH21	Negative	Negative	Negative
Gyps africanus	white-backed vultures	Unknown	MO45	KH22	Negative	Negative	Negative
Gyps africanus	white-backed vultures	Unknown	533723	KH23	Negative	Negative	Negative
Gyps africanus	white-backed vultures	Unknown	P069	KH24	Negative	Negative	Negative
Gyps africanus	white-backed vultures	Unknown	2007	KH25	Negative	Negative	Negative

Bird Species	Common Name	Location	Sample Code	Data Code	Plasmodium	Haemoproteus	Leucocytozoon
Gyps africanus	white-backed vultures	Unknown	632995	KH26	Negative	Negative	Negative
Gyps africanus	white-backed vultures	Unknown	633744	KH27	Negative	Negative	Negative
Gyps africanus	white-backed vultures	Unknown	2000	KH28	Negative	Negative	Negative
Gyps africanus	white-backed vultures	Unknown	A270	KH29	Negative	Negative	Negative
Gyps africanus	white-backed vultures	Unknown	M048	KH30	Negative	Negative	Negative
Gyps africanus	white-backed vultures	Unknown	63299	KH31	Negative	Negative	Negative
Gyps africanus	white-backed vultures	Unknown	532931	KH32	Negative	Negative	Negative
Gyps africanus	white-backed vultures	Unknown	A276	KH33	Negative	Negative	Negative
Gyps africanus	white-backed vultures	Unknown	633752	KH34	Negative	Negative	Negative
Gyps africanus	white-backed vultures	Unknown	D336	KH35	Negative	Negative	Negative
Gyps africanus	white-backed vultures	Unknown	632993	KH36	Negative	Negative	Negative
Gyps africanus	white-backed vultures	Unknown	633751	KH37	Negative	Negative	Negative
Gyps africanus	white-backed vultures	Unknown	5004	KH38	Negative	Negative	Negative
Gyps africanus	white-backed vultures	Unknown	7028	KH39	Negative	Negative	Negative
Gyps africanus	white-backed vultures	Unknown	A271	KH40	Negative	Negative	Negative
Gyps africanus	white-backed vultures	Unknown	526	KH41	Negative	Negative	Negative

Bird Species	Common Name	Location	Sample Code	Data Code	Plasmodium	Haemoproteus	Leucocytozoon
Gyps africanus	white-backed vultures	Unknown	633759	KH42	Negative	Negative	Negative
Gyps africanus	white-backed vultures	Unknown	A278	KH43	Negative	Negative	Negative
Gyps africanus	white-backed vultures	Unknown	A281	KH44	Negative	Negative	Negative
Gyps africanus	white-backed vultures	Unknown	2004	KH45	Negative	Negative	Negative
Gyps africanus	white-backed vultures	Unknown	532557	KH46	Negative	Negative	Negative
Gyps africanus	white-backed vultures	Unknown	2007	KH47	Negative	Negative	Negative
Gyps africanus	white-backed vultures	Unknown	633481	KH48	Negative	Negative	Negative
Gyps africanus	white-backed vultures	Unknown	532910	KH49	Negative	Negative	Negative
Gyps africanus	white-backed vultures	Unknown	5011	KH50	Negative	Negative	Negative
Gyps africanus	white-backed vultures	Unknown	5006	KH51	Negative	Negative	Negative
Gyps africanus	white-backed vultures	Unknown	2001	KH52	Negative	Negative	Negative
Gyps africanus	white-backed vultures	Unknown	533765	KH53	Negative	Negative	Negative
Gyps africanus	white-backed vultures	Unknown	2002	KH54	Negative	Negative	Negative
Gyps africanus	white-backed vultures	Unknown	632994	KH55	Negative	Negative	Negative
Gyps africanus	white-backed vultures	Unknown	A280	KH56	Negative	Negative	Negative
Gyps africanus	white-backed vultures	Unknown	5007	KH57	Negative	Negative	Negative

Bird Species	Common Name	Location	Sample Code	Data Code	Plasmodium	Haemoproteus	Leucocytozoon
Gyps africanus	white-backed vultures	Unknown	533759	KH58	Negative	Negative	Negative
Gyps africanus	white-backed vultures	Unknown	2009	KH59	Negative	Negative	Negative
Gyps africanus	white-backed vultures	Unknown	4269	KH60	Negative	Negative	Negative
Gyps africanus	white-backed vultures	Unknown	051E	KH61	Negative	Negative	Negative
Gyps africanus	white-backed vultures	Unknown	5016	KH62	Negative	Negative	Negative
Gyps africanus	white-backed vultures	Unknown	5005	KH63	Negative	Negative	Negative
Gyps africanus	white-backed vultures	Unknown	H041	KH64	Negative	Negative	Negative
Gyps africanus	white-backed vultures	Unknown	H031	KH65	Negative	Negative	Negative
Gyps africanus	white-backed vultures	Unknown	H039	KH66	Negative	Negative	Negative
Gyps africanus	white-backed vultures	Unknown	A245	KH67	Negative	Negative	Negative
Gyps africanus	white-backed vultures	Unknown	A250	KH68	Negative	Negative	Negative
Gyps africanus	white-backed vultures	Unknown	P067	KH69	Negative	Negative	Negative
Gyps africanus	white-backed vultures	Unknown	P066	KH70	Negative	Negative	Negative
Bubo africanis	spotted eagle owl	Unknown	SE01	FH1	Negative	Negative	Negative
Bubo africanis	spotted eagle owl	Unknown	SE02	FH2	Negative	Negative	Negative
Bubo africanis	spotted eagle owl	Unknown	SE03	FH3	Negative	Negative	Negative

Bird Species	Common Name	Location	Sample Code	Data Code	Plasmodium	Haemoproteus	Leucocytozoon
Bubo africanis	spotted eagle owl	Unknown	SE04	FH4	Negative	Negative	Negative
Bubo africanis	spotted eagle owl	Unknown	SE05	FH5	Negative	Positive (H18)	Negative
Stephanoaetus coronatus	crowned eagle	Unknown	CE A5	FH6	Negative	Negative	Negative
Stephanoaetus coronatus	crowned eagle	Unknown	CE A6	FH7	Negative	Negative	Negative
Stephanoaetus coronatus	crowned eagle	Unknown	CE A7	FH8	Negative	Negative	Negative
Gyps coprotheres	Cape vulture	Rhino and Lion Nature Reserve,	8171	MH1	Negative	Negative	Negative
Gyps coprotheres	Cape vulture	Neemalt, Freestate	4363	MH2	Negative	Negative	Negative
Gyps coprotheres	Cape vulture	Skeerpoort colony	7987	MH3	Negative	Negative	Negative
Gyps coprotheres	Cape vulture	Rehab from Thabazimbi	43405	MH4	Negative	Negative	Negative
Gyps coprotheres	Cape vulture	Skeerpoort Colony, Skeerpoort	38406	MH5	Negative	Negative	Negative
Gyps coprotheres	Cape vulture	Rhino and Lion Nature Reserve,	8170	MH6	Negative	Negative	Negative
Gyps coprotheres	Cape vulture	Skeerpoort colony	47022	MH7	Negative	Negative	Negative
Gyps coprotheres	Cape vulture	Rhino and Lion Nature Reserve, Johannesburg	9504	MH8	Negative	Negative	Negative
Gyps coprotheres	Cape vulture	Rhino and Lion Nature Reserve,	8304	MH9	Negative	Negative	Negative
Gyps coprotheres	Cape vulture	Rhino and Lion Nature Reserve,	8300	MH10	Negative	Negative	Negative
Gyps coprotheres	Cape vulture	Rhino and Lion Nature Reserve,	8452	MH11	Negative	Negative	Negative
Gyps coprotheres	Cape vulture	Ventersdorp	4349	MH12	Negative	Negative	Negative

Bird Species	Common Name	Location	Sample Code	Data Code	Plasmodium	Haemoproteus	Leucocytozoon
Gyps coprotheres	Cape vulture	Kransberg, Thabazimbi	3673	MH13	Negative	Positive*	Negative
Gyps coprotheres	Cape vulture	Rhino and Lion Nature Reserve,	8460	MH14	Negative	Negative	Negative
Gyps coprotheres	Cape vulture	Skeerpoort colony	47021	MH15	Negative	Negative	Negative
Gyps coprotheres	Cape vulture	Nyoka Ridge, Skeerpoort	10233	MH16	Negative	Negative	Negative
Gyps coprotheres	Cape vulture	Nyoka Ridge, Skeerpoort	10231	MH17	Negative	Negative	Negative
Gyps coprotheres	Cape vulture	Skeerpoort colony	47064	MH18	Negative	Negative	Negative
Gyps coprotheres	Cape vulture	Rhino and Lion Nature Reserve,	8453	MH19	Negative	Negative	Negative
Gyps coprotheres	Cape vulture	Rhino and Lion Nature Reserve,	8456	MH20	Negative	Positive*	Negative
Gyps coprotheres	Cape vulture	Skeerpoort colony	47024	MH21	Negative	Negative	Negative
Gyps coprotheres	Cape vulture	Skeerpoort colony	47063	MH22	Negative	Negative	Negative
Gyps coprotheres	Cape vulture	Rhino and Lion Nature Reserve,	8311	MH23	Negative	Negative	Negative
Gyps coprotheres	Cape vulture	Skeerpoort colony	47042	MH24	Negative	Negative	Negative
Gyps coprotheres	Cape vulture	Skeerpoort colony	47041	MH25	Negative	Negative	Negative
Gyps coprotheres	Cape vulture	Skeerpoort colony	47045	MH26	Negative	Negative	Negative
Gyps coprotheres	Cape vulture	Skeerpoort colony	47052	MH27	Negative	Negative	Negative
Gyps coprotheres	Cape vulture	Rhino and Lion Nature Reserve,	8455	MH28	Negative	Negative	Negative
Gyps coprotheres	Cape vulture	Skeerpoort colony	47040	MH29	Negative	Negative	Negative
Gyps coprotheres	Cape vulture	Rhino and Lion Nature Reserve,	8457	MH30	Negative	Negative	Negative
Gyps coprotheres	Cape vulture	Rhino and Lion Nature Reserve,	8287	MH31	Negative	Negative	Negative
Gyps coprotheres	Cape vulture	Skeerpoort colony	47054	MH32	Negative	Negative	Negative
Gyps coprotheres	Cape vulture	Rhino and Lion Nature Reserve,	8172	MH33	Negative	Negative	Negative

Bird Species	Common Name	Location	Sample Code	Data Code	Plasmodium	Haemoproteus	Leucocytozoon
Gyps coprotheres	Cape vulture	Rhino and Lion Nature Reserve,	8054	MH34	Negative	Negative	Negative
Gyps coprotheres	Cape vulture	Rhino and Lion Nature Reserve,	8454	MH35	Negative	Positive*	Negative
Gyps coprotheres	Cape vulture	Nooitgedacht	38402	MH36	Negative	Negative	Negative
Gyps coprotheres	Cape vulture	Rhino and Lion Nature Reserve,	8458	MH37	Negative	Negative	Negative
Gyps coprotheres	Cape vulture	Rhino and Lion Nature Reserve,	8293	MH38	Negative	Positive*	Negative
Gyps coprotheres	Cape vulture	Nyoka Ridge, Skeerpoort	10230	MH39	Negative	Negative	Negative
Gyps coprotheres	Cape vulture	Skeerpoort colony	7989	MH40	Negative	Negative	Negative
Gyps coprotheres	Cape vulture	Skeerpoort colony	47025	MH41	Negative	Negative	Negative
Gyps coprotheres	Cape vulture	Skeerpoort colony	47061	MH42	Negative	Negative	Negative
Gyps coprotheres	Cape vulture	Hoedspruit	6670	MH43	Negative	Negative	Negative
Gyps coprotheres	Cape vulture	Rehab from Limpopo Province	43398	MH44	Negative	Negative	Negative
Gyps coprotheres	Cape vulture	Skeerpoort Colony, Skeerpoort	10229	MH45	Negative	Negative	Negative
Gyps coprotheres	Cape vulture	Rhino and Lion Nature Reserve,	8438	MH46	Negative	Negative	Negative
Gyps coprotheres	Cape vulture	Nyoka Ridge, Skeerpoort	10228	MH47	Negative	Negative	Negative
Gyps coprotheres	Cape vulture	Ntsikeni Wildlife Reserve	12	CH1	Negative	Positive*	Negative
Gyps coprotheres	Cape vulture	Ntsikeni Wildlife Reserve	29	CH2	Negative	Negative	Negative
Gyps coprotheres	Cape vulture	Ntsikeni Wildlife Reserve	13	CH3	Negative	Negative	Negative
Gyps coprotheres	Cape vulture	Ntsikeni Wildlife Reserve	23	CH4	Negative	Negative	Negative

Bird Species	Common Name	Location	Sample Code	Data Code	Plasmodium	Haemoproteus	Leucocytozoon
Gyps coprotheres	Cape vulture	Ntsikeni Wildlife Reserve	41	CH5	Negative	Negative	Negative
Gyps coprotheres	Cape vulture	Ntsikeni Wildlife Reserve	43	CH6	Negative	Negative	Negative
Gyps coprotheres	Cape vulture	Ntsikeni Wildlife Reserve	46	CH7	Negative	Negative	Negative
Gyps coprotheres	Cape vulture	Ntsikeni Wildlife Reserve	1	CH8	Negative	Negative	Negative
Gyps coprotheres	Cape vulture	Ntsikeni Wildlife Reserve	2	CH9	Negative	Negative	Negative
Gyps coprotheres	Cape vulture	Ntsikeni Wildlife Reserve	44	CH10	Negative	Negative	Negative
Gyps coprotheres	Cape vulture	Kokstad	14	CH11	Negative	Positive	Negative
Gyps coprotheres	Cape vulture	Kokstad	16	CH12	Negative	Negative	Negative
Gyps coprotheres	Cape vulture	Kokstad	22	CH13	Negative	Negative	Negative
Gyps coprotheres	Cape vulture	Kokstad	39	CH14	Negative	Negative	Negative
Gyps coprotheres	Cape vulture	Kokstad	47	CH15	Negative	Negative	Negative
Gyps coprotheres	Cape vulture	Kokstad	33	CH16	Negative	Negative	Negative
Gyps coprotheres	Cape vulture	Kokstad	19	CH17	Negative	Negative	Negative
Gyps coprotheres	Cape vulture	Kokstad	28	CH18	Negative	Negative	Negative
Gyps coprotheres	Cape vulture	Kokstad	10	CH19	Negative	Negative	Negative
Gyps coprotheres	Cape vulture	Kokstad	17	CH20	Negative	Negative	Negative
Gyps coprotheres	Cape vulture	Kokstad	37	CH21	Negative	Negative	Negative
Gyps coprotheres	Cape vulture	Kokstad	32	CH22	Negative	Negative	Negative
Gyps coprotheres	Cape vulture	Kokstad	75	CH23	Negative	Negative	Negative

Table S2.3 : Genbank information of added raptor sequences

Accession number	Species name	Host and Phylogeny code	Network Code	Genus of Parasite
MK390804	*Athene brama*	Spotted owlet 1	H2	*Haemoproteus*

MT281476	*Falco tinnunculus*	Common kestrel 1	H1	*Haemoproteus*
MT281465	*Accipiter gularis*	Japanese sparrowhawk 1	H3	*Haemoproteus*
MT281464	*Tyto alba*	Barn owl 2	H5	*Haemoproteus*
MT281480	*Accipiter nisus*	Eurasian sparrowhawk 4	H4	*Haemoproteus*
MK390809	*Tyto alba*	Barn owl 3	H7	*Haemoproteus*
MT281469	*Accipter nisus*	Eurasian eagle owl 4	H6	*Haemoproteus*
MG192533	*Ibycter americanus*	Red throated caracara 1	H17	*Haemoproteus*
EU627838	*Tyto alba*	Barn owl 4	H22	*Haemoproteus*
MT281472	*Accipter nisus*	Eurasian sparrowhawk 5	H20	*Haemoproteus*
MT281485	*Accipter nisus*	Eurasian sparrowhawk 6	H19	*Haemoproteus*
MT281466	*Accipter nisus*	Eurasian sparrowhawk 7	H18	*Haemoproteus*
MT281463	*Accipiter francesiae*	France's sparrowhawk 2	H21	*Haemoproteus*
EU627829	*Tyto alba*	Barn owl 8	H23	*Haemoproteus*
MF621945	*Falco sparverius*	American kestrel 2	H23	*Haemoproteus*
MF621946	*Falco sparverius*	American kestrel 3	H23	*Haemoproteus*
MT281477	*Falco amurensis*	Amur falcon 58	H41	*Haemoproteus*
MT281475	*Falco amurensis*	Amur falcon 59	H32	*Haemoproteus*
MF621947	*Falco sparverius*	American kestrel 4	H40	*Haemoproteus*
EF564177	*Falco naumanni*	Lesser kestrel 5	H32	*Haemoproteus*
MN224231	*Buteo buteo*	Common buzzard 2	H12	*Haemoproteus*

Table S2.4: MalAvi information of added raptor sequences

MalAvi sequence code	Species name	Host and Phylogeny code	Genus of parasite	Network code
MILANS02	*Milvus migrans*	Black kite 4	*Haemoproteus*	H16
MILANS01	*Milvus migrans*	Black kite 3	*Haemoproteus*	H10
MILANS03	*Milvus migrans*	Black kite 5	*Haemoproteus*	H11
ALARV01	*Bubo bubo*	Eurasian eagle owl 1	*Haemoproteus*	H14
ALARV02	*Bubo bubo*	Eurasian eagle owl 2	*Haemoproteus*	H15
ALARV03	*Bubo bubo*	Eurasian eagle owl 3	*Haemoproteus*	H13
BNOW02	*Tyto alba*	Barn owl 5	*Haemoproteus*	H22
BNOW03	*Tyto alba*	Barn owl 6	*Haemoproteus*	H25
BNOW01	*Falco naumanni*	Barn owl 7	*Haemoproteus*	H24
LK02	*Falco naumanni*	Lesser kestrel 2	*Haemoproteus*	H40
LK03	*Falco naumanni*	Lesser kestrel 3	*Haemoproteus*	H42
LK04	*Falco naumanni*	Lesser kestrel 4	*Haemoproteus*	H41
LK01	*Falco naumanni*	Lesser kestrel 1	*Haemoproteus*	H41
ASOT06	*Asio otus*	Long eared 0wl 3	*Leucocytozoon*	H17
ASOT2	*Asio otus*	Long eared owl 2	*Leucocytozoon*	H17
BUVIR03	*Aegolius acadicus*	Northern saw-wet owl 3	*Leucocytozoon*	H16
GLSJO02	*Glaucidium sjostedti*	Sjöstedt's barred owlet 1	*Leucocytozoon*	H19
BUVIR04	*Aegolius acadicus*	Northern saw-wet owl 4	*Leucocytozoon*	H23
BUVIR06	*Aegolius acadicus*	Northern saw-wet owl 6	*Leucocytozoon*	H20
BUVIR05	*Aegolius acadicus*	Northern saw-wet owl 5	*Leucocytozoon*	H21
AEFUN02	*Aegolius funereus*	Boreal owl 1	*Leucocytozoon*	H15
OTSCO01	*Otus scops*	Eurasian scops owl 1	*Leucocytozoon*	H12
OTSCO03	*Otus scops*	Eurasian scops owl 2	*Leucocytozoon*	H13
ASOT1	*Asio otus*	Long eared owl 1	*Leucocytozoon*	H22
BUVIR02	*Aegolius acadicus*	Northern saw-wet owl 2	*Leucocytozoon*	H16
CIAE02	*Circus aeruginosus*	Western Marsh harrier	*Leucocytozoon*	H25
BNOW04	*Tyto alba*	Barn owl 1	*Leucocytozoon*	H26
AEMO02	*Aeygpius monachus*	Cinerious vulture 1	*Leucocytozoon*	H27
BUVIR01	*Aegolius acadicus*	Northern saw-wet owl 1	*Leucocytozoon*	H11

MalAvi sequence code	Species name	Host and Phylogeny code	Genus of parasite	Network code
MILVUS02	*Milvus migrans*	Black kite 2	*Leucocytozoon*	H10
ACNI02	*Accipiter nisus*	Eurasian sparrowhawk 2	*Leucocytozoon*	H9
ACNI01	*Accipiter nisus*	Eurasian sparrowhawk 1	*Leucocytozoon*	H7
ACNI03	*Accipiter nisus*	Eurasian sparrowhawk 3	*Leucocytozoon*	H8
ACCBRE02	*Accipiter brevipes*	Levant sparrowhawk 1	*Leucocytozoon*	H3
ACCBRE03	*Accipiter brevipes*	Levant sparrowhawk 2	*Leucocytozoon*	H2
ACCFRA01	*Accipiter francesiae*	France's sparrowhawk 1	*Leucocytozoon*	H1
MILVUS01	*Milvus migrans*	Black kite 1	*Leucocytozoon*	H5
BUBT2	*Buteo buteo*	Common buzzard 1	*Leucocytozoon*	H6
ACNI04	*Accipiter nisus*	Eurasian sparrowhawk 4	*Leucocytozoon*	H4

Table S3.1: Raw microsatellite data of the 178 Amur falcons (*Falco amurensis*) in this study.

Sample	FalVes13		FalVes28		FalVes38		FalVes31		FalVes43		FalVes05		Bbu03		FalVes26	
MR714	112	112	279	263	288	236	104	104	211	211	225	197	204	?	?	?
MR722	106	106	335	263	258	240	132	124	203	203	237	225	204	204	191	207
MR715	106	106	299	227	258	248	128	136	203	203	237	229	204	204	199	199
MR725	106	109	279	235	242	240	140	124	211	211	225	201	204	206	203	193
MR733	106	106	347	279	250	242	132	128	203	203	225	225	202	196	205	201
MR785	106	106	335	263	246	224	124	120	219	215	197	225	206	206	199	199
MR810	100	109	263	259	240	230	120	120	203	199	205	177	206	206	207	201
MR740	106	109	295	243	236	224	140	124	203	203	229	229	202	202	207	199
MR820	106	106	319	267	232	262	132	132	219	207	249	229	204	204	201	199
MR822	106	121	315	219	220	220	128	124	199	199	237	233	204	204	203	205
MR818	106	106	235	331	236	232	?	?	?	?	?	?	204	197	209	199
MR825	106	109	330	271	262	240	132	128	211	211	221	197	204	204	197	197
MR821	103	106	333	319	250	236	124	136	203	203	229	205	204	196	197	197
MR827	106	106	263	219	246	242	136	136	219	203	225	221	204	204	203	203
MR728	106	109	287	263	234	224	124	132	215	215	233	197	202	202	207	199
MR826	106	106	287	279	258	242	132	108	211	211	233	177	204	204	209	199

Sample	FalVes13		FalVes28		FalVes38		FalVes31		FalVes43		FalVes05		Bbu03		FalVes26	
MR756	106	109	335	271	228	224	124	108	215	203	229	217	204	204	205	199
MR823	106	109	331	271	262	240	132	128	211	211	221	197	204	204	199	199
MR753	106	106	235	251	242	236	128	108	219	211	253	241	206	196	205	199
MR745	106	106	267	227	246	236	136	132	215	207	229	217	204	196	199	193
MR824	106	106	263	251	254	232	132	120	207	207	233	209	202	206	203	207
MR761	106	106	295	267	242	228	136	132	211	207	229	217	204	204	207	199
MR819	106	106	275	247	250	236	132	108	211	199	225	217	204	204	207	199
MR775	106	112	323	247	252	230	160	140	203	199	245	233	204	204	205	199
MR718	106	106	351	263	316	232	136	132	207	203	233	225	204	204	199	199
MR754	106	106	355	243	246	232	124	108	203	203	229	229	204	204	199	199
MR762	106	109	303	247	240	225	132	108	199	199	261	205	204	204	207	203
MR782	106	109	319	227	246	228	136	120	223	223	?	?	206	204	199	203
MR760	106	106	287	263	286	236	132	132	219	219	237	233	204	204	205	199
MR776	106	106	275	355	238	228	124	124	207	199	237	229	204	204	203	203
MR751	106	106	263	215	246	228	144	140	207	207	245	209	202	196	205	189
MR774	106	106	279	263	250	240	128	108	219	203	229	225	204	204	205	205
MR746	106	106	299	243	240	224	136	116	211	203	221	217	204	204	203	199
MR765	103	106	295	287	240	236	140	124	219	211	249	229	204	202	209	199
MR783	106	121	235	287	242	236	136	132	211	211	197	201	204	204	209	199
MR768	106	106	227	327	258	228	?	?	?	?	?	?	204	196	213	205
MR752	106	106	283	279	246	242	124	108	211	203	189	181	204	204	213	193
MR748	115	115	263	251	242	228	128	120	203	203	237	233	204	204	207	207
MR757	106	106	223	267	242	234	136	132	203	203	237	233	204	196	209	199
MR755	106	109	199	267	232	232	132	108	203	203	217	205	204	204	203	199
MR779	103	106	295	231	240	224	136	136	203	203	225	217	202	198	211	211
MR747	106	106	299	351	260	228	132	132	203	203	229	217	204	204	207	199
MR780	103	103	243	247	232	234	132	136	203	203	233	233	204	204	205	205
MR755	106	106	331	259	246	228	140	116	203	199	241	229	204	198	197	191
MR807	106	106	263	251	254	232	132	120	207	207	233	209	204	196	203	203
MR769	103	106	283	231	236	232	120	108	211	211	245	225	204	204	205	205
MR772	103	106	263	263	232	228	132	124	219	215	237	229	206	204	205	199
MR750	106	106	263	263	228	224	116	116	203	203	201	201	204	204	205	205
MR764	103	106	199	199	232	232	108	108	211	211	189	181	202	202	203	203

Sample	FalVes13		FalVes28		FalVes38		FalVes31		FalVes43		FalVes05		Bbu03		FalVes26	
MR759	115	115	235	235	250	250	116	124	219	219	237	233	206	206	209	205
N468	106	106	367	303	250	228	128	124	219	211	221	193	206	206	203	199
N214	106	109	271	271	250	240	136	132	211	203	249	213	206	203	203	199
N594	106	127	255	275	288	246	120	120	211	211	229	225	206	206	205	205
N886	106	106	287	355	242	242	108	108	203	203	245	229	206	206	205	199
N23	106	109	275	271	250	240	132	120	211	203	237	237	206	196	211	205
N610	106	106	?	?	?	?	144	136	215	215	237	237	204	196	199	199
N904	106	127	299	295	236	226	132	124	203	203	233	197	204	204	205	199
N352	106	106	223	251	236	220	132	108	215	215	225	225	206	197	205	199
N484	106	127	251	247	236	258	136	124	203	203	193	185	204	204	207	199
N233	106	109	339	267	232	224	136	124	215	203	241	213	?	?	?	?
N305	106	127	255	371	254	236	136	132	211	211	245	233	204	202	205	199
N575	106	109	219	215	250	240	136	108	215	207	225	197	206	206	205	199
N317	106	106	267	235	246	224	144	140	231	223	?	?	204	204	199	199
N605	106	109	315	243	252	244	132	108	211	211	233	221	204	204	203	199
N9	106	127	299	235	250	250	132	120	211	203	233	229	204	204	197	201
N499	106	127	243	239	284	246	132	124	203	195	241	233	204	204	199	199
N257	106	127	271	259	242	236	132	124	207	207	229	221	206	198	205	201
N33	106	109	239	231	232	224	144	132	207	203	241	197	204	204	205	199
N26	106	106	263	215	290	242	132	132	203	199	233	225	206	204	199	197
N356	106	106	227	215	?	?	136	128	211	211	245	205	206	204	207	199
N925	106	109	267	251	254	250	152	136	207	203	209	205	204	204	203	197
N599	106	109	299	291	238	232	124	108	211	211	245	225	204	204	203	199
N461	106	106	347	323	268	236	132	124	203	199	237	201	204	204	203	199
N602	106	127	267	335	242	224	132	128	215	215	221	197	204	204	203	193
N890	106	127	275	263	242	240	108	132	215	215	233	217	206	206	205	197
N3	109	130	255	251	228	224	136	108	211	203	245	225	206	204	205	199
N224	106	106	263	239	242	234	136	120	203	203	241	209	206	206	209	205
N6	106	109	307	299	228	224	128	108	215	207	217	205	204	202	211	205
N486	106	106	319	287	242	236	128	132	207	207	225	217	204	204	207	203
N885	106	109	307	279	?	?	132	128	207	199	225	217	?	?	?	?
N490	106	106	367	299	250	228	128	124	219	211	221	193	204	204	207	199
N913	106	106	287	283	242	232	132	124	203	203	249	225	204	196	199	199

Sample	FalVes13		FalVes28		FalVes38		FalVes31		FalVes43		FalVes05		Bbu03		FalVes26	
N857	100	106	335	279	288	242	128	108	227	207	225	209	204	204	209	199
N136	106	109	283	271	254	254	132	124	219	219	205	177	204	196	205	199
N597	100	109	355	311	268	232	132	120	?	?	?	?	204	204	203	199
N494	106	106	359	243	238	236	136	132	203	203	237	233	204	206	207	199
N134	106	106	319	263	236	232	132	104	219	219	189	181	204	204	205	203
N894	106	127	291	247	290	236	132	128	211	203	237	225	204	204	205	205
N48	106	112	279	247	244	228	136	124	219	215	219	205	204	204	203	203
N10	106	118	371	299	?	?	136	124	203	203	237	221	204	204	197	197
N355	106	100	275	263	240	236	144	128	219	211	237	233	206	206	199	199
N553	106	106	259	255	240	240	144	108	211	203	209	197	202	202	199	199
N211	106	130	323	231	290	242	128	108	203	199	229	197	204	204	203	199
N1149	106	106	227	215	246	232	136	128	211	211	245	205	206	206	?	?
N308	106	106	375	223	240	234	140	124	203	203	225	217	204	202	205	199
N551	106	127	347	319	250	232	128	124	207	203	189	197	206	204	207	203
N590	106	127	291	295	288	246	140	124	219	219	197	189	204	198	207	203
N254	106	127	287	235	224	224	152	132	211	207	237	225	204	204	203	197
711	106	109	295	275	288	242	124	108	203	203	197	189	204	204	205	205
168	100	100	335	279	254	236	128	108	219	207	225	209	204	204	199	197
125	106	106	283	235	246	236	128	124	219	203	221	217	206	202	205	205
665	106	106	303	239	254	238	132	124	215	203	237	257	204	206	199	197
N315	106	106	301	279	254	250	136	108	203	195	233	217	204	204	209	197
N628	106	106	299	243	246	242	124	108	207	207	237	229	206	206	215	199
N390	106	106	291	219	258	230	136	124	203	203	233	221	204	204	199	199
N393	106	106	279	259	?	?	136	108	203	203	233	217	204	204	203	199
N324	106	109	263	263	242	234	120	120	203	195	225	221	204	204	207	203
N354	106	106	335	223	232	228	136	132	207	207	233	225	204	204	207	203
N335	106	109	279	231	246	236	124	108	199	199	233	197	204	204	199	193
N569	106	106	319	223	288	242	132	124	215	215	229	221	204	204	199	197
157	100	106	335	279	238	224	128	108	227	207	225	209	204	204	203	195
N212	106	106	359	335	246	236	108	108	207	207	241	205	204	204	207	199
N210	106	106	303	239	246	236	132	124	215	203	237	225	204	204	203	197
N230	106	106	303	239	242	232	132	124	215	203	237	225	204	204	199	195
N203	106	106	287	263	242	242	132	124	203	203	249	197	204	204	207	203

Sample	FalVes13		FalVes28		FalVes38		FalVes31		FalVes43		FalVes05		Bbu03		FalVes26	
N219	106	109	259	259	246	242	128	108	227	207	225	209	204	204	207	207
N239	106	106	291	219	236	232	136	108	203	195	233	217	204	204	199	199
N614	106	109	279	231	242	232	136	124	203	203	233	221	204	204	201	199
N860	106	106	287	263	258	230	?	?	?	?	?	?	204	204	205	199
663	106	106	279	259	232	228	136	132	207	207	233	225	204	204	201	199
607	106	106	263	223	246	236	124	108	199	199	233	197	206	206	199	195
167	106	106	319	223	238	224	132	132	215	215	229	221	204	204	205	197
631	106	106	359	335	242	228	136	132	203	203	237	217	204	204	207	197
636	106	106	267	215	242	228	136	132	203	203	237	217	204	204	211	207
684	106	106	267	215	242	220	140	120	203	203	241	229	204	204	209	209
703	106	109	251	247	258	238	136	108	219	219	229	221	204	204	209	199
6	106	100	307	231	288	224	136	124	203	203	229	205	204	204	199	197
592	106	106	283	279	246	242	132	132	219	203	233	203	204	204	203	197
N347	106	106	363	279	224	224	132	124	219	219	205	177	204	204	205	199
N320	106	106	227	199	254	230	132	132	219	219	233	225	204	204	211	199
N365	106	106	247	227	232	224	136	132	207	207	221	177	204	204	199	193
N911	106	109	243	243	228	222	128	124	203	195	241	221	204	204	203	199
N896	106	127	275	263	242	230	144	124	207	231	217	217	202	202	205	?
N377	106	106	319	319	240	230	136	108	219	203	237	205	204	204	203	195
677	106	106	315	259	258	238	136	120	219	203	237	197	206	206	199	205
666	106	106	323	271	242	228	136	120	203	203	233	197	204	204	209	211
593	106	106	319	283	232	220	132	108	211	211	257	237	204	204	209	199
105	106	157	331	331	232	226	136	132	203	203	229	221	204	204	199	199
186	106	100	275	251	258	230	160	120	211	207	221	201	204	204	211	199
199	106	106	247	243	254	228	140	108	207	203	225	217	204	204	203	199
71	106	106	347	295	242	230	136	128	207	203	245	205	204	204	199	199
127	106	106	323	243	246	232	124	108	211	207	233	197	204	204	207	209
111	106	106	315	259	242	232	132	128	207	203	245	177	204	204	207	189
589	100	106	235	343	?	?	128	108	203	203	229	221	204	204	203	203
334	106	106	355	259	246	224	132	124	203	203	241	225	204	204	203	199
691	106	106	287	235	242	224	144	132	231	223	205	193	204	204	199	199
N645	106	109	355	295	242	232	132	128	203	203	245	217	204	204	215	199
538	106	109	275	271	250	240	?	?	?	?	205	193	204	204	211	199

Sample	FalVes13		FalVes28		FalVes38		FalVes31		FalVes43		FalVes05		Bbu03		FalVes26	
646	106	106	291	291	246	240	124	108	215	215	245	238	204	204	205	201
N375	106	106	303	291	234	220	136	120	215	207	197	189	204	204	199	201
N389	106	106	255	215	?	?	?	?	?	?	245	177	204	204	191	197
N387	106	106	235	287	258	236	136	124	203	203	197	189	204	204	207	203
N191	?	?	?	?	250	236	132	124	211	199	233	225	204	204	211	209
N741	106	109	319	231	242	228	132	132	203	195	225	217	204	204	205	199
N351	106	106	283	275	224	224	124	124	211	199	245	233	204	204	199	191
N357	106	106	275	243	258	236	132	128	211	203	237	205	204	204	191	191
N893	106	106	303	231	236	236	128	128	203	203	237	233	204	204	209	207
N595	106	106	287	287	242	228	136	132	203	203	245	237	204	204	211	199
184	106	97	319	315	242	232	140	108	203	203	233	221	204	204	211	197
602	106	118	267	251	228	228	132	132	215	215	245	217	204	204	199	191
681	106	106	331	279	246	230	132	124	203	195	237	229	204	204	205	205
242	106	106	259	215	254	236	132	124	203	195	237	225	204	204	199	199
188	106	106	239	235	250	236	152	132	203	203	225	213	204	204	203	203
N231	106	106	283	235	254	236	128	124	219	203	221	217	204	204	203	203
N1150	106	106	263	215	242	236	108	108	211	203	237	209	204	204	199	199
N40	100	106	331	223	242	224	152	124	215	203	237	221	204	204	199	199
N1082	106	106	291	259	242	224	132	124	203	199	237	197	204	204	199	199
N572	106	106	283	235	240	236	132	124	211	211	197	189	204	204	207	207
N205	106	106	287	235	240	228	132	108	211	203	245	233	204	204	199	199
N250	106	106	283	271	236	225	136	128	211	203	237	197	204	204	203	203
N554	106	109	263	231	246	240	124	108	203	203	241	233	204	204	197	197
202	106	106	299	271	?	?	136	132	203	203	229	205	204	204	203	203
140	106	106	243	235	250	232	140	120	207	207	233	181	204	204	207	197
144	?	?	?	?	236	236	132	132	203	203	209	201	204	204	215	199
91	106	106	323	239	236	228	132	132	215	215	229	197	204	204	207	199
175	106	106	327	259	236	228	124	108	219	203	233	229	204	204	203	199
42	106	106	251	251	236	236	136	124	215	211	245	213	204	204	207	199
117	106	106	263	259	242	236	136	136	211	203	237	229	204	196	199	197
N335	106	109	279	231	246	236	124	108	199	199	233	197	204	204	199	193
N569	106	106	319	223	288	242	132	124	215	215	229	221	204	204	199	197
157	100	106	335	279	238	224	128	108	227	207	225	209	204	204	203	195

Table S3.2 : Pairwise Nei's Genetic distance estimates for the Mooi River and Newcastle populations.

Locality	Mooi River	Newcastle
Mooi River	0.00	
Newcastle	0.03	0.00

Table S3.3: The Evanno table output from STRUCTURE analyses

K	Reps	Mean LnP(K)	Stdev LnP(K)	Ln'(K)	|Ln"(K)|	Delta K
2	10	-6481.73	147.77	NA	NA	NA
3	10	-6547.82	282.26	-66.09	841.90	2.98
4	10	-7455.81	2177.2386	-907.99	976.36	0.45
5	10	-7387.44	1447.42	68.37	NA	NA

Table S3.4: Proportion of membership (Q values) of each geographic region under admixture model with correlated allele frequencies. The inferred genetic clusters are four (K = 4).

	Inferred genetic clusters			
	1	2	3	4
Mooi River	0.239	0.252	0.263	0.246
Newcastle	0.255	0.253	0.250	0.242

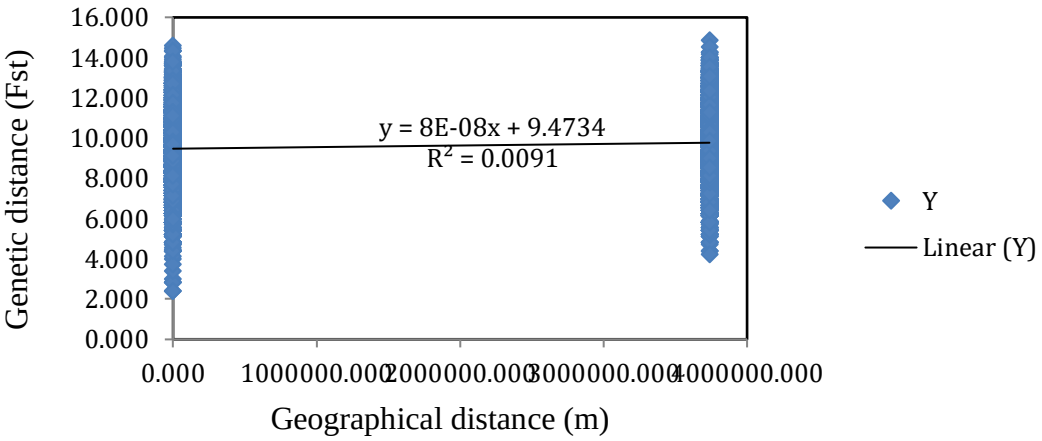

Figure S3.1: Relationship between FST estimates against geographic distance from Mantel test for the 178 Amur falcon individuals grouped by the two roosting sites. R = 0.01

Table S4.1:Heterozygous indices calculad in GENHET.

Sample	**PHt**	**Ho**	**SH**	**IR**	**HL**
MR714	0,50	0,67	0,62	0,46	0,42
MR722	0,63	0,93	0,83	0,10	0,26
MR715	0,50	0,74	0,66	0,27	0,40

Sample	PHt	Ho	SH	IR	HL
MR725	0,88	1,30	1,16	-0,07	0,13
MR733	0,63	0,93	0,83	0,20	0,35
MR785	0,63	0,93	0,83	0,21	0,27
MR810	0,75	1,11	0,99	0,18	0,21
MR740	0,63	0,93	0,83	0,24	0,35
MR820	0,63	0,93	0,83	0,13	0,27
MR822	0,63	0,93	0,83	0,21	0,35
MR818	0,80	1,27	1,14	-0,14	0,12
MR825	0,63	0,93	0,83	0,20	0,33
MR821	0,75	1,11	0,99	0,06	0,26
MR827	0,50	0,74	0,66	0,29	0,41
MR728	0,75	1,11	0,99	0,11	0,19
MR826	0,63	0,93	0,83	0,12	0,26
MR756	0,88	1,30	1,16	-0,18	0,07
MR823	0,63	0,93	0,83	0,16	0,33
MR753	0,88	1,30	1,16	-0,08	0,07
MR745	0,88	1,30	1,16	-0,15	0,07
MR824	0,75	1,11	0,99	0,08	0,20
MR761	0,75	1,11	0,99	-0,06	0,13
MR819	0,75	1,11	0,99	-0,05	0,13
MR775	0,88	1,30	1,16	-0,12	0,07
MR718	0,63	0,93	0,83	0,08	0,27
MR754	0,38	0,56	0,50	0,44	0,55
MR762	0,75	1,11	0,99	0,05	0,19
MR782	0,86	1,35	1,17	-0,06	0,15
MR760	0,50	0,74	0,66	0,29	0,40
MR776	0,50	0,74	0,66	0,32	0,41
MR751	0,75	1,11	0,99	0,11	0,20
MR774	0,63	0,93	0,83	0,13	0,27
MR746	0,75	1,11	0,99	-0,06	0,13
MR765	1,00	1,48	1,33	-0,22	0,00
MR783	0,75	1,11	0,99	0,01	0,19
MR768	0,80	1,27	1,14	-0,09	0,12
MR752	0,75	1,11	0,99	-0,03	0,13
MR748	0,50	0,74	0,66	0,37	0,40
MR757	0,75	1,11	0,99	-0,03	0,20
MR755	0,63	0,93	0,83	0,13	0,35
MR779	0,63	0,93	0,83	0,26	0,40
MR747	0,50	0,74	0,66	0,27	0,40
MR780	0,38	0,56	0,50	0,52	0,55
MR755	0,88	1,30	1,16	-0,10	0,07
MR807	0,63	0,93	0,83	0,19	0,33
MR769	0,63	0,93	0,83	0,19	0,33
MR772	0,88	1,30	1,16	-0,11	0,16
MR750	0,13	0,19	0,17	0,83	0,85
MR764	0,25	0,37	0,33	0,71	0,78
MR759	0,38	0,56	0,50	0,59	0,57
N468	0,75	1,11	0,99	0,07	0,13
N214	0,88	1,30	1,16	-0,05	0,16

Sample	PHt	Ho	SH	IR	HL
N594	0,50	0,74	0,66	0,42	0,47
N886	0,38	0,56	0,50	0,51	0,55
N23	0,88	1,30	1,16	-0,04	0,15
N610	0,33	0,56	0,48	0,52	0,70
N904	0,75	1,11	0,99	-0,04	0,19
N352	0,63	0,93	0,83	0,22	0,35
N484	0,75	1,11	0,99	-0,01	0,19
N233	1,00	1,34	1,24	-0,19	0,00
N305	0,88	1,30	1,16	-0,10	0,13
N575	0,88	1,30	1,16	-0,04	0,07
N317	0,57	0,90	0,78	0,19	0,32
N605	0,75	1,11	0,99	0,01	0,19
N9	0,75	1,11	0,99	0,02	0,22
N499	0,75	1,11	0,99	-0,01	0,20
N257	0,88	1,30	1,16	-0,02	0,13
N33	0,88	1,30	1,16	-0,17	0,07
N26	0,75	1,11	0,99	-0,03	0,21
N356	0,71	1,11	0,98	0,04	0,23
N925	0,88	1,30	1,16	-0,13	0,07
N599	0,75	1,11	0,99	0,01	0,19
N461	0,75	1,11	0,99	-0,07	0,13
N602	0,75	1,11	0,99	0,04	0,19
N890	0,75	1,11	0,99	0,12	0,19
N3	1,00	1,48	1,33	-0,21	0,00
N224	0,63	0,93	0,83	0,21	0,26
N6	1,00	1,48	1,33	-0,20	0,00
N486	0,63	0,93	0,83	0,13	0,26
N885	1,00	1,40	1,28	-0,20	0,00
N490	0,75	1,11	0,99	-0,03	0,13
N913	0,63	0,93	0,83	0,12	0,33
N857	0,88	1,30	1,16	-0,12	0,07
N136	0,75	1,11	0,99	0,08	0,28
N597	0,83	1,25	1,15	-0,06	0,09
N494	0,75	1,11	0,99	-0,04	0,20
N134	0,63	0,93	0,83	0,15	0,26
N894	0,75	1,11	0,99	0,00	0,20
N48	0,75	1,11	0,99	0,05	0,20
N10	0,57	0,89	0,78	0,22	0,39
N355	0,75	1,11	0,99	0,10	0,20
N553	0,50	0,74	0,66	0,37	0,42
N211	0,88	1,30	1,16	-0,15	0,07
N1149	0,57	0,85	0,77	0,29	0,30
N308	0,75	1,11	0,99	-0,01	0,20
N551	1,00	1,48	1,33	-0,24	0,00
N590	0,88	1,30	1,16	-0,04	0,13
N254	0,75	1,11	0,99	0,03	0,22
711	0,63	0,93	0,83	0,16	0,33
168	0,75	1,11	0,99	0,08	0,13
125	0,75	1,11	0,99	0,06	0,20

Sample	PHt	Ho	SH	IR	HL
665	0,88	1,30	1,16	-0,17	0,07
N315	0,75	1,11	0,99	-0,02	0,13
N628	0,63	0,93	0,83	0,22	0,26
N390	0,50	0,74	0,66	0,26	0,40
N393	0,57	0,89	0,78	0,12	0,31
N324	0,63	0,93	0,83	0,19	0,36
N354	0,63	0,93	0,83	0,13	0,26
N335	0,75	1,11	0,99	0,03	0,19
N569	0,63	0,93	0,83	0,13	0,26
157	0,88	1,30	1,16	-0,09	0,07
N212	0,50	0,74	0,66	0,31	0,40
N210	0,75	1,11	0,99	-0,06	0,13
N230	0,75	1,11	0,99	-0,08	0,13
N203	0,50	0,74	0,66	0,27	0,41
N219	0,63	0,93	0,83	0,20	0,36
N239	0,63	0,93	0,83	0,10	0,27
N614	0,75	1,11	0,99	-0,03	0,19
N860	0,60	0,96	0,86	0,04	0,23
663	0,63	0,93	0,83	0,12	0,26
607	0,63	0,93	0,83	0,22	0,26
167	0,50	0,74	0,66	0,31	0,40
631	0,63	0,93	0,83	0,10	0,26
636	0,63	0,93	0,83	0,10	0,26
684	0,50	0,74	0,66	0,31	0,40
703	0,75	1,11	0,99	0,03	0,19
6	0,75	1,11	0,99	-0,01	0,19
592	0,63	0,93	0,83	0,11	0,27
N347	0,50	0,74	0,66	0,31	0,41
N320	0,50	0,74	0,66	0,30	0,40
N365	0,63	0,93	0,83	0,14	0,26
N911	0,75	1,11	0,99	0,01	0,23
N896	0,71	1,07	0,96	0,19	0,25
N377	0,63	0,93	0,83	0,14	0,29
677	0,75	1,11	0,99	0,06	0,13
666	0,63	0,93	0,83	0,12	0,26
593	0,63	0,93	0,83	0,13	0,26
105	0,50	0,74	0,66	0,30	0,49
186	0,88	1,30	1,16	-0,10	0,07
199	0,75	1,11	0,99	-0,06	0,13
71	0,63	0,93	0,83	0,10	0,27
127	0,75	1,11	0,99	-0,02	0,13
111	0,75	1,11	0,99	-0,04	0,13
589	0,57	0,89	0,78	0,21	0,39
334	0,63	0,93	0,83	0,08	0,26
691	0,63	0,93	0,83	0,14	0,27
N645	0,75	1,11	0,99	-0,03	0,19
538	0,83	1,22	1,13	-0,12	0,09
646	0,50	0,74	0,66	0,33	0,42
N375	0,75	1,11	0,99	0,00	0,13

Sample	PHt	Ho	SH	IR	HL
N389	0,60	0,94	0,86	0,11	0,23
N387	0,63	0,93	0,83	0,11	0,26
N191	0,83	1,23	1,07	-0,08	0,09
N741	0,75	1,11	0,99	-0,02	0,21
N351	0,50	0,74	0,66	0,31	0,42
N357	0,63	0,93	0,83	0,14	0,27
N893	0,38	0,56	0,50	0,47	0,55
N595	0,50	0,74	0,66	0,27	0,42
184	0,75	1,11	0,99	0,01	0,19
602	0,50	0,74	0,66	0,35	0,49
681	0,63	0,93	0,83	0,12	0,27
242	0,63	0,93	0,83	0,08	0,27
188	0,50	0,74	0,66	0,28	0,40
N231	0,63	0,93	0,83	0,13	0,27
N1150	0,50	0,74	0,66	0,26	0,41
N40	0,75	1,11	0,99	-0,01	0,20
N1082	0,63	0,93	0,83	0,08	0,27
N572	0,50	0,74	0,66	0,31	0,40
N205	0,63	0,93	0,83	0,08	0,27
N250	0,63	0,93	0,83	0,12	0,27
N554	0,63	0,93	0,83	0,16	0,33
202	0,43	0,67	0,59	0,35	0,47
140	0,63	0,93	0,83	0,17	0,26
144	0,33	0,49	0,43	0,53	0,63
91	0,50	0,74	0,66	0,29	0,40
175	0,75	1,11	0,99	-0,08	0,13
42	0,50	0,74	0,66	0,30	0,44
117	0,75	1,11	0,99	0,00	0,20

Haemoproteus Data

Table S4.2: Results of a mixed-effects logistic regression with the continuous factor proportion of heterozygous loci.

	Estimate	Standard error	Statistical value (Z)	P-value
Intercept	-1.71	0.76	-2.25	**0.03**
Age	-0.23	0.33	-0.70	0.49
Sex	0.19	0.32	0.58	0.56
Proportion of Heterozygous loci	1.59	1.10	1.45	0.15

Table S4.3: Results of a mixed-effects logistic regression with the continuous factor observed heterozygosity.

	Estimate	Standard error	Statistical value (Z)	P value

Intercept	-1.75	0.77	-2.27	**0.02**
Age	-0.22	0.33	-0.68	0.50
Sex	0.19	0.32	0.58	0.56
Observed Heterozygosity	1.11	0.75	1.48	0.14

Table S4.4: Results of a mixed-effects logistic regression with the continuous factor standardized heterozygosity**.**

	Estimate	**Standard error**	**Statistical value (Z)**	**P-value**
Intercept	-1.73	0.77	-2.27	0.02
Age	-0.22	0.33	-0.68	0.50
Sex	0.19	0.32	0.58	0.56
Standardized Heterozygosity	1.22	0.83	1.48	0.14

Table S4.5: Results of a mixed-effects logistic regression with the continuous factor of internal relatedness.

	Estimate	**Standard error**	**Statistical value (Z)**	**P-value**
Intercept	-0.51	0.29	-1.73	0.08
Age	-0.23	0.33	-0.71	0.48
Sex	0.19	0.32	0.58	0.56
Internal relatedness	-1.18	0.92	-1.28	0.20

Table S4.6: Results of a mixed-effects logistic regression with the continuous factor homozygosity by locus.

	Estimate	**Standard error**	**Statistical value (Z)**	**P-value**
Intercept	-0.26	0.43	-0.61	0.54
Age	-0.23	0.33	-0.70	0.49
Sex	0.18	0.32	0.56	0.58
Homozygosity by locus	-1.45	1.17	-1.24	0.22

Lineage A

Table S4.7: Results of a mixed-effects logistic regression with the continuous factor proportion of heterozygous loci.

	Estimate	Standard error	Statistical value (Z)	P-value
Intercept	-2.38	0.85	-2.80	**0.01**
Age	-0.50	0.36	-1.39	0.17
Sex	0.12	0.34	0.58	0.56
Proportion of Heterozygous loci	2.17	1.21	1.79	0.07*

Table S4.8: Results of a mixed-effects logistic regression with the continuous factor observed heterozygosity.

	Estimate	Standard error	Statistical value (Z)	P-value
Intercept	-2.46	0.87	-2.83	**<0.01**
Age	-0.49	0.36	-1.37	0.17
Sex	0.12	0.35	0.36	0.72
Observed Heterozygosity	1.52	0.83	1.84	0.07*

Table S4.9: Results of a mixed-effects logistic regression model run on Lineage A data with the continuous factor internal relatedness.

	Estimate	Standard error	Statistical value (Z)	P-value
Intercept	-0.76	0.31	-2.44	**0.01**
Age	-0.50	0.36	-1.38	0.17
Sex	0.13	0.35	0.38	0.70
Internal relatedness	-1.47	1.02	-1.44	0.15

Lineage B

Table S4.10: Results of a mixed-effects logistic regression with the continuous factor proportion of heterozygous loci.

	Estimate	Standard error	Statistical value (Z)	P-value
Intercept	-2.48	1.55	-1.60	0.11
Age	0.20	0.70	0.29	0.78
Sex	0.88	0.74	1.18	0.24
Proportion of Heterozygous loci	-1.58	2.35	-0.67	0.50

Table S4.11: Results of a mixed-effects logistic regression with the continuous factor observed heterozygosity.

	Estimate	**Standard error**	**Statistical value (Z)**	**P-value**
Intercept	-2.41	1.56	-1.55	**0.12**
Age	0.20	0.70	0.28	0.78
Sex	0.88	0.74	1.19	0.24
Observed Heterozygosity	-1.13	1.60	-0.71	0.48

Table S4.12: Results of a mixed-effects logistic regression model run on Lineage B data with the continuous factor internal relatedness. Significant values are in bold and approaching significance values are indicated with a *.

	Estimate	**Standard error**	**Statistical value (Z)**	**P-value**
Intercept	-2.42	1.55	-1.56	0.12
Age	0.20	0.70	0.29	0.78
Sex	0.88	0.74	1.19	0.24
Internal relatedness	-1.25	1.78	-0.71	0.48