AVIAN HAEMOSPORIDIAN DYNAMICS IN THE WESTERN GHATS SKY ISLAND BIRD SYSTEM

by

POOJA GUPTA

(Under the Direction of Guha Dharmarajan and James C. Beasley)

ABSTRACT

Parasites are ubiquitous, abundant, and functionally significant components of ecosystems. Parasites and the diseases they cause can have deleterious effects on organisms at all scales of biological organization. Avian haemosporidians (genera *Plasmodium* and *Haemoproteus*) are a diverse group of vector-borne blood parasites that affect bird populations globally, with epidemic mortalities in Hawaiian and New Zealand birds. However, we still lack an understanding of avian haemosporidian infection dynamics in the Western Ghats, a global biodiversity hotspot, in India. This mountain chain is topographically complex, hosts one of the most globally isolated Sky Island systems and harbors remarkable bird diversity, with many endemic and threatened bird species. Here, I examined for the first time, eco-evolutionary factors affecting avian haemosporidian dynamics in the Western Ghats Sky Island system. Specifically, I elucidated diversity, host range, phylogeography and community structure of avian haemosporidians. I also tested the utility of novel genomic tools using sequence capture approach to advance parasite genomics and comparative phylogenomics.

My dissertation research revealed several novel haemosporidian parasite lineages endemic to the Western Ghats. *Haemoproteus* lineages were host specialists and co-speciated

with their bird hosts whereas *Plasmodium* were generalists. Community structure of *Haemoproteus* was more affected by host phylogeny/ecology whereas *Plasmodium* were affected by geographic barriers, potentially leading to greater likelihood of emergence by *Plasmodium* in novel host communities. Variation in infection risk among bird communities was affected by host ecological traits promoting parasite exposure (e.g., sociality, foraging strata) and traits associated with host susceptibility (e.g., sexual dimorphism, body condition). Additionally, host phylogeny contributed substantially in predicting *Haemoproteus* infection risk compared to *Plasmodium*, reiterating that host ecology and host phylogeny together influence infection dynamics. Overall, my dissertation research provides novel insights into how geographical barriers, host (e.g. host phylogeny/ecology) and parasite (e.g. host specificity) factors influence avian haemosporidian dynamics. This has broad implications for understanding disease dynamics in natural populations and important from wildlife health perspective. In my final chapter, I demonstrated that sequence capture is a promising approach to obtain high-quality parasite genomic data and gain unique insights into the evolution of avian haemosporidians, an exciting advancement in avian haemosporidian research.

INDEX WORDS: Apicomplexa, *Haemoproteus*, *Plasmodium*, avian malaria, Western Ghats, India, disease emergence, phylogenetics, community structure, ultraconserved elements, genomics

AVIAN HAEMOSPORIDIAN DYNAMICS IN THE WESTERN GHATS SKY ISLAND BIRD SYSTEM

by

POOJA GUPTA

B.S., University of Jabalpur, India, 2008

M.S., University of Madras, India, 2010

A Dissertation Submitted to the Graduate Faculty of The University of Georgia in Partial Fulfillment of the Requirements for the Degree

DOCTOR OF PHILOSOPHY

ATHENS, GEORGIA

2020

AVIAN HAEMOSPORIDIAN DYNAMICS IN THE WESTERN GHATS SKY ISLAND BIRD SYSTEM

by

POOJA GUPTA

Major Professor:	Guha Dharmarajan,
	James C. Beasley
Committee:	Michael J. Yabsley
	Travis C. Glenn
	V.V. Robin

Electronic Version Approved:

Ron Walcott
Interim Dean of the Graduate School
The University of Georgia
August 2020

DEDICATION

In the loving memory of my Grandfather, Rajaram Asati, and his son, my Uncle, Ajay Asati, who always supported and encouraged me to follow my dreams.

Be mindful when it comes to your words. A string of some that don't mean much to you, may stick with someone else for a lifetime. –Rachel Wolchin

ACKNOWLEDGEMENTS

My dissertation research on bird parasites originated back in India and has since travelled with me to the States. My journey had been challenging, yet an interesting roller coaster and would not have been possible without the support of many people. I owe great thanks to my advisor, Guha Dharmarajan, for not only offering his support and guidance throughout my PhD project; but also showing confidence in me initially and giving me the opportunity to continue working with him, when he moved from India to USA. He introduced me to the fascinating world of parasites and the field of disease ecology, and for that I am very grateful.

I would also like to thank my co-advisor James Beasley, my committee members, Travis Glenn, Michael Yabsley and Robin Vijayan for their valuable support, expertise and help to improve the research presented here. Michael provided most of the bird samples for my NGS work (Chapter 5) and Travis generously offered space in his lab to kick start the NGS project, and for that I am extremely thankful.

I also owe a tremendous amount to mentors and friends at NCBS, my pre-PhD lab. The time spent in Uma Ramakrishnan's lab benefitted tremendously in preparing myself for graduate school and gave me a sense of belonging in academia. Her incredible enthusiasm and passion for science and education continues to inspire me and her skilled mentorship helped me grow as a scientist. Thanks to Robin Vijayan, who introduced me to the Sky Islands, that instantly made me fall in love with the system and inspired me to work on the disease aspect of Sky Island birds. He also introduced me to my current advisor, Guha Dharmarajan, which was a very fortunate co-incidence and presented a right opportunity at the right time. Robin had always been a great

friend and mentor to me. His pep talks have always helped and still help boost my confidence. I will forever be grateful to you, for giving me my first research opportunity, teaching me invaluable life-lessons and helping to forge my career path. I feel very fortunate to have met such great mentors and experience the good side of academia during my time at NCBS. I would not be doing science if it were not for your support in the early stages of my career.

This work would not have been possible without the herculean effort of many researchers, volunteers and field assistants, more people than I can possibly list here, who helped me collect bird samples in the field. I would like to especially thank C.K. Vishnudas and the field crew particularly Eswaran, Jagadish and Seetharaman for working tirelessly and assisting with sample collection from the remote locations in the Western Ghats to support my research. I am also thankful to Anuradha Chandramouli for her assistance in screening the bird samples for parasite detection. I am grateful as well to Troy Kieran and Marissa Howard, who helped me conduct NGS library preparation in Travis' lab and were always available for troubleshooting in the lab or helping with genomics data analysis.

My time at SREL was made easier by the support and friendship of Erik Neff, Wes Dixon, Amelia Russell and other SREL colleagues, including Lucy and Vicky. I am also thankful to Dina Rasquinha, who provided me my second home on my trips to UGA campus for my research, after I moved to SREL.

My dissertation research was funded by U.S. Department of Energy Financial Assistance Award no. DE-EM0004391, for which I am thankful. I would also like to thank the staff and officers from Tamil Nadu, Kerala and Karnataka forest departments for granting the permits to work in these protected areas. I am thankful for the support from Warnell School of Forestry and

Natural Sciences for the Graduate Assistantship and the travel award that allowed me to attend the Evolution meeting and build networks with peers in the scientific community.

I could not have completed my PhD without the unwavering love and support of my parents, Anand Gupta and Seema Gupta, my brother Anshul Gupta and my sister-in-law, Preeti Gupta. Coming from a family where no one has ever travelled abroad before, it was very hard for my parents to truly understand my passion for science and to let me pursue my PhD in a different continent, thousands of miles away from them. But I am very thankful to them for trusting me with my choices (good and bad ones) and providing their unconditional support throughout this journey. I will never be able to repay you for all that you sacrificed for me, but I hope that I have made you proud. My in-laws, Ajay Singh Chauhan and Kalpana Chauhan, provided much needed encouragement and were always understanding when I missed important occasions in my family. Getting married half-way through my PhD and not being able to meet them afterwards was not something they would have expected from their daughter-in-law, but I am forever grateful to them, for giving me the space and freedom to pursue my dreams.

Finally, I owe my biggest thanks to my better half, my loving husband, Rahul Chauhan, for his utmost serenity and being my pillar of strength. His profound belief in me and unfailing encouragement (or critique sometimes) kept me motivated throughout and helped me sustain through graduate school. He did everything, from travelling 12 hours and changing multiple flights to see me in a remote town, spending nights remotely troubleshooting my Python code errors, to proofreading my manuscripts and dissertation. He also made me a better science communicator by listening to my endless rattle on my research. He helped me see the positive on tough days and celebrated with me on my good days, that helped me keep going. He always provided a unique, effective perspective on my self-doubts and kept me sane when things started

going haywire towards the end of my graduate school. I truly would not have survived through the struggles and challenges of graduate school without your love, care and incessant support.

TABLE OF CONTENTS

Page

LIST OF FIGURES

Page

CHAPTER 1

INTRODUCTION AND LITERATURE REVIEW

Emerging infectious diseases (EIDs) are infections caused by newly identified pathogens or those caused by previously known pathogens that are rapidly increasing in incidence and/or geographic range (re-emerging) (Morse, 1995). Disease emergence in natural populations can be impacted by myriad ecological factors, including land use changes, host and parasite diversity, and environmental modifications such as natural or anthropogenic climate change (Altizer et al., 2013; Harvell et al., 2002; Johnson et al., 2013; Lafferty, 2009; Ostfeld and Keesing, 2012). Additionally, other factors, such as host defense mechanisms (e.g., resistance vs. tolerance; (Ayres and Schneider, 2012; Råberg et al., 2007), host evolutionary history (Parker et al., 2015), host life-history traits (Isaksson et al., 2013; Johnson et al., 2012) may also influence emergence of infectious diseases. Consequently, increase in the emergence/re-emergence of infectious diseases may be driven by the complex interplay between various eco-evolutionary and environmental factors (Morse, 1995; Patz et al., 2000; Schrag and Wiener, 1995).

Understanding emerging infectious diseases (EIDs) in wildlife is important because about 70% of infectious diseases that have emerged in humans in the last few decades are zoonotic (Altizer et al., 2013; Harvell et al., 2002; Jones et al., 2008; Smith et al., 2009). Additionally, many EIDs directly impact individual health, population dynamics, as well as ecosystem structure and function. The increased rate of emergence is especially pronounced in the cases of diseases transmitted by arthropod vectors, such as mosquitoes and ticks, and these diseases are a major cause of concern in human and wildlife populations (Christou, 2011; Gubler, 1998;

Kilpatrick and Randolph, 2012; Sakkas et al., 2016). It has been estimated that vector-borne diseases constitute about 28% of emerging infectious diseases (Jones et al., 2008) and contribute to about 17% of the global human infectious disease burden (WHO, 2016). Additionally, the introduction of vector-borne diseases in novel geographic areas, especially islands, have often resulted in serious issues for the native wildlife (e.g. population decline, extinction).

Vector-borne diseases introduced by exotic vectors and/or hosts represent novel epidemiological scenario, with dramatic negative consequences, for native species. For instance, endemic rats *Rattus macleari* and *R. nativitas* disappeared from Christmas Island in the Indian Ocean due to the introduction of trypanosome pathogen from black rats (*R. rattus*) which is transmitted by fleas (Pickering and Norris 1996, (Wyatt et al., 2008). The mass extirpation of native Hawaiian honeycreepers infected by *Plasmodium* spp. in the late 19th century is a classic example of a vector-borne EID due to the introduction of mosquito vector *Culex quinquefasciatus* (Atkinson et al., 2000; Lapointe et al., 2012; Warner, 1968). *Plasmodium* spp. has also been reported in the loss of endemic avian fauna and bird mortalities in New Zealand, primarily due to the introduction of malaria infected hosts (Alley et al., 2010; Howe et al., 2012; Schoener et al., 2014).

Avian malaria – a vector-borne disease caused by protozoan parasites belonging to genera *Plasmodium* and other related haemosporidians such as *Haemoproteus* and *Parahaemoproteus* is an important emerging disease in bird populations globally. Parasite transmission between avian hosts occurs primarily via several genera of mosquitoes in the case of *Plasmodium*, Culicoides biting midges and louse flies in the case of *Haemoproteus* and *P*arahaemoproteus (Valkiŭnas, 2005). In my dissertation, I propose to examine avian

haemosporidian dynamics in bird communities inhabiting the Sky Islands in the Western Ghats, Southern India.

1. Study system

1.1. Western Ghats

The Western Ghats of India, together with Sri Lanka, constitutes one of the 34 global biodiversity hotspot and one of the four in the Indian subcontinent (including the Himalayas, Indo-Burma and Sundaland hotspots) (Myers et al., 2000). The Western Ghats is an ancient mountain range, approximately 1600 km long, that runs parallel to the west coast of Peninsular India (about 30 to 50 kms inland) extending between 8° to 21°N. The Western Ghats are remarkably biodiverse and support about 30% of all flora and fauna in less than 6% of the total land area of India (CEPF, 2007). However, this ecoregion is severely threatened by climate change and anthropogenic disturbances, with human population density higher than all other global hotspots and anthropogenic fragmentation, including deforestation and land-use conversion (e.g., for agriculture, timber plantations). Consequently, only 20% of the original forest cover remains in the region (Cincotta et al., 2000; Sloan et al., 2014).

The Western Ghats mountains have a complex geological history and are located on a faulted edge of the Deccan plateau (Briggs et al., 2003). They originated as a series of uplifts during the movement of the Indian plate after the break-up of the super continent of Gondwana between 150-50 mya (Chatterjee et al., 2013). The Western Ghats are a geologically complex biogeographical region which have undergone changes in its topography and climate, during the Quarternary climatic oscialltions (glacial-interglacial periods) since 2.5 mya (Sukumar et al., 1993). Such climatic events have shaped the distribution and genetic structure of several extant organisms in the Western Ghats, including bird species. The origin of Western Ghats flora and

fauna, and especially birds are poorly understood, and two general hypotheses have been proposed. One of the commonly accepted hypotheses is the long-distance dispersal of species from the Himalayas. It has been hypothesized that when the climate was cooler, during mid Miocene, forests were more contiguous in the Indian subcontinent and wet tropical forests occurred in Central India, which facilitated dispersal of species from Himalayas to the Western Ghats (Karanth, 2003). Another proposed hypothesis is vicariance, which suggests that forests in the Western Ghats became isolated when India collided with the Asian plate. This led to the onset of drier periods and subsequent replacement of tropical evergreen forests by dry deciduous forests in the Central part of India. However, tropical montane areas in the Western Ghats likely maintained stable local climatic conditions (during glacial-interglacial cycles) and served as glacial refugia (Prasad et al., 2009). This probably led to persistence of ancient lineages and subsequent diversification of specialized endemic lineages in the Western Ghats (Joshi and Karanth, 2013). Thus, Western Ghats are often referred to as both museum and cradle of biodiversity.

Topography and climate

The Western Ghats are interrupted by three major bio-geographical breaks, the Chaliyar River valley (2-3 km wide), the deepest Palghat Gap (40km wide,) and the Shencottah Gap (10 km wide). The Palghat and Shencottah gaps are ancient fissures that originated about 500 mya, as a result of the combined effect of shearing and erosion (D'Cruz et al., 2000; Soman et al., 1990). Several studies have shown the effect of these gaps on population genetic structure of a range of taxa including mammals, birds, reptiles, amphibians (Robin et al., 2015b; Vidya et al., 2005; Vijayakumar et al., 2016). Such patterns of genetic differentiation in hosts could impact genetic structure in parasite populations harboring these bird hosts.

The western slopes of the Western Ghats mountains receive heavy annual rainfall (average 3000-4000 mm) while the eastern slopes are drier (average 500-1000 mm annually). The higher rainfall on the western slopes is predominantly due to the southwest monsoon from June to September. Additionally, rainfall decreases from south to north. The climate is characterized by low mean annual temperatures compared to surrounding plains and greater diurnal temperature variation.

1.2. Shola Sky Islands

The Western Ghats hosts one of the most isolated Sky Island systems of the world (Myers et al., 2000). Sky islands are high elevation habitats isolated by drastically different lowlands. The Western Ghats Sky Islands harbor exceptional biodiversity and disproportionately higher number of endemic species. These Sky Islands occur above 1400 m, and are home to a unique, natural mosaic of forests and grasslands, locally known as *Shola* habitats. The *Sholas* are comprised of tropical evergreen montane forests with stunted trees, in the sheltered valleys and folds of the mountains interspersed by vast expanses of grasslands on mountain tops while low elevations harbor drier habitats The *Shola* forests are typically covered in mist and are characterized as tropical montane cloud forests consisting of stunted trees and host a variety of mosses and epiphytes. The montane wet forests likely acted as refugia for many species as surrounding areas increasingly become drier (see above).

Shola-grassland mosaic

The montane forests and grasslands mosaic have existed for more than 20,000 years but contracted and expanded over geologic time scales in response to fluctuating changes in global climate (Sukumar et al., 1995, 1993). The dynamic equilibrium of forest-grassland mosaic has primarily been influenced by multiple cycles of wet and dry periods. During the Pleistocene (2.5

mya to 11.5 kya), cold, drier periods and low CO_2 levels led to contraction of montane forests while expansion of forests occurred during warm, wetter periods (Meher-Homji, 1967; Sukumar et al., 1995, 1993). Based on the stable carbon isotope analysis by Sukumar et al. (1995), changes in historical climate shaped vegetation type and consequently impacted the structure and composition of the montane ecosystem. Shifts in C4/C3 vegetation types likely occurred during the late Quaternary period which were associated with changes in moisture levels and atmospheric CO2 (see table below). For example, during last glacial maxima (20-18kya), low atmospheric CO2, low rainfall and low mean temperatures led to predominance of C4 grasslands. Subsequently, during the Interglacial period (18-10 kya), high global CO2, high rainfall due to strong southwest monsoon and high mean temperatures resulted in expansion of C3 vegetation and montane forests in the Western Ghats. In the last 3000-6000 years, weakening of southwest monsoon and increased human activities have reduced rainfall and led to shifts from C3 to C4 vegetation and decline of montane forests(Sukumar et al., 1995).

Two competing hypotheses have been historically proposed for the current extant of the forest-grassland mosaic in the Western Ghats. First, frost hypothesis (Meher-Homji, 1967, 1965; Ranganathan, 1938) suggests that frosts coupled with dry spell confines the woody species (forest trees) to the valleys and folds of hills because tropical species cannot withstand frost-induced stress and cannot establish themselves in open grasslands. This has been supported by the observation that open grassland species are temperate or subtropical in origin whereas the forest tree species in the *Shola* interiors are tropical in origin. However, it was later argued that the effect of frost would be higher in the valleys as cold air is heavier and will tend to accumulate more in the valleys, preventing the establishment of forests. Second, the fire hypothesis (Gupta, 1960; Noble, 1967) suggests that the grasslands originated as a result of

frequent burnings from agricultural and pastoral activities introduced by early human settlers and fire has been important in maintaining grasslands.

While a single limiting factor may not adequately explain the presence of forest-grassland mosaic, a recent study by Das et al. (2015), showed that interaction between topographic and bioclimatic factors were important in determining the extant distribution of forests and grasslands in the Western Ghats shola-grassland mosaics. Furthermore, Joshi et al. (2019) conducted an experimental study and showed that the low temperatures and frost during winters kills and restricts the establishment of native tree seedlings in grasslands; consequently, maintaining the dynamic equilibrium of forest-grassland mosaic. The authors further suggested that forest-grassland mosaic has been naturally maintained by climatic conditions. Increased temperatures in the light of global climate change might support the expansion of tree species and allow spread of forests into the montane grasslands; consequently, affecting the distribution of flora and fauna inhabiting the Western Ghats Sky Islands.

2. Shola Sky Islands and avian haemosporidian dynamics

The replicated arrangement of geographically discrete, identical habitats may serve as an ideal natural laboratory to explore eco-evolutionary dynamics underlying avian haemosporidian infection risk for many reasons. First, the Western Ghats Sky Islands form one of the world's biodiversity hotspots (Myers et al., 2000) with a diverse array of endemic species, especially in the *Sholas* (Robin and Nandini, 2012). The diverse avifauna in this ecosystem consists of altitudinal or habitat specialists which are restricted to the *Sholas,* and generalists whose distributions span widely from the lowlands to high elevation habitats. A myriad of ecological (habitat and host ecological traits) and environmental factors (climate) within each Sky Island may affect distribution and range of parasites and influence the eco-evolutionary dynamics of

avian haemosporidian parasites. Additionally, the high diversity of hosts may provide more opportunities for host switching and subsequent lineage diversification (Hayakawa et al., 2008). Unlike single species studies, studying an entire native bird community will help to get a holistic understanding of mechanisms of parasite diversification and parasite community structure.

Second, spatial heterogeneity due to host and habitat variation may influence disease ecological dynamics. On a large spatial scale, two major biogeographic divides- Palaghat gap and Shencottah gap (about 100km and 60 km wide, respectively) have facilitated the genetic divergence of many Sky Island birds (Robin et al., 2015b) and may serve as bridges/barriers to parasite populations. On a small spatial scale, the Sky Island habitat is naturally fragmented due to the mosaic of forest and grasslands patches resulting in disjunct host distribution. Furthermore, anthropogenic modification of about 80% of the habitat due to plantations and agricultural fields have led to disjunct distribution of Sky Island bird populations (Robin et al., 2015a, 2014). Habitat fragmentation may also impact vector abundance and likely alter ecological dynamics of avian haemosporidian parasites (Chasar et al., 2009; Laurance et al., 2013). Thus, Western Ghats Sky Island system offers an exciting opportunity to explore the role of spatial factors in avian haemosporidian dynamics at multiple spatial scales.

Third, climate change perturbations may be more pronounced in the Western Ghats tropical montane community compared to temperate counterparts, which may differently affect avian haemosporidian dynamics. Tropical species generally exhibit low environmental tolerance; thus, in response to global climate changes, their geographical ranges may either shift upwards in elevation or undergo range collapse due to unavailability of suitable habitat (Chen et al., 2011; Colwell et al., 2008; Fortini et al., 2015; Peh, 2007; Seimon et al., 2007). Tropical montane species, in particular, are the most susceptible to the global climate change due to their narrow

geographical ranges, high endemism and limited dispersal opportunities (Sorte and Jetz 2010; Freeman and Freeman 2014). The effects of climate change in the Western Ghats montane community may further be exacerbated due to the topography of the mountains. As the mountain top surface area declines with elevation, habitat availability reduces and limits the possibility of upslope shifts for montane species (Elsen and Tingley, 2015). In addition to effects of global climate change on host distribution, local environmental variations with high rainfall, humidity and low temperatures at higher elevations may alter the geographic range of avian haemosporidian parasites. The development of both haemosporidian parasites and vectors are highly sensitive to temperature and other climatic conditions but the effects may vary for *Plasmodium* and *Haemoproteus* parasites (Garamszegi, 2011; Pérez-Rodríguez et al., 2014). Thus, global and local environmental changes may disrupt the established interactions and provide more opportunities for novel pathogens to emerge in novel hosts.

Examining avian haemosporidian dynamics in the Western Ghats Sky Island system provides a novel opportunity to understand the interaction between mountain topography, disease and climate change, and help us to predict disease incidence patterns in future climate change scenarios. Despite the vulnerability of montane birds due to habitat fragmentation and climate change, malaria parasite prevalence and eco-evolutionary dynamics in the Western Ghats Sky Island community remains unexplored. Hence, studies examining contribution of pathogens to the existing threat for endemic fauna in this tropical continental archipelago are highly warranted and will be an important step towards their conservation.

3. Objectives

My dissertation examines avian haemosporidian disease dynamics in the Western Ghats Sky Island bird communities with the goal of understanding the relative role of different

evolutionary and ecological factors that may influence avian haemosporidian dynamics. Additionally, I optimized novel genomic tools using target sequence capture approach to obtain genome wide parasite data that can potentially accelerate ecological and evolutionary research of avian haemosporidian parasites.

The first chapter of my dissertation, *Towards a more healthy conservation paradigm: Integrating disease and molecular ecology to aid biological conservation*, emphasizes the role of disease ecology in biological conservation. This chapter provides a broad overview of how genetic and genomic tools have contributed towards an improved understanding of ecological and evolutionary processes that affect disease dynamics across scales of biological organization. Parasites are ubiquitous and integral members of ecosystems, comprising a significant proportion of total biomass in many ecosystems. Although parasites contribute to myriad ecological, evolutionary and ecosystem-level processes, we are yet to fully recognize the functional role of parasites in ecological systems. Consequently, viewing disease as an eco-evolutionary process is important for understanding parasite transmission and management of parasites in natural populations, in order to aid biological conservation. This chapter serves as a foundation for the development of theoretical framework and concepts tested in subsequent chapters of my dissertation.

Chapter Two, *Geographic and host species barriers differentially affect generalist and specialist parasite community structure in a tropical Sky Island archipelago*, examines the eco-evolutionary mechanisms that influence the diversity and distribution of avian haemosporidians in the Western Ghats. Here, I explored the relative importance of geographic (e.g., spatial distance and biogeographic gaps), climatic (e.g., elevational gradients) and host species barriers (e.g., host phylogeny and host ecology) in driving avian haemosporidian parasite community

structure. Understanding such eco-evolutionary differences among parasites can provide critical insights into infectious disease emergence in naive host communities.

Chapter Three, *Host phylogeny matters: Examining sources of variation in infection risk by blood parasites across a tropical montane bird community in India*, employs a phylogenetic approach to elucidate the role of host ecological and morphological traits that may influence avian haemosporidian dynamics in the Western Ghats Sky Island system. Host ecological traits that increase hosts exposure to parasites and/or host susceptibility may affect disease prevalence across hosts and influence epidemiology of avian haemosporidian parasites. Identifying the ecological factors that shape variation in infection risk among host species is important to understand infectious disease dynamics in wildlife populations.

Chapter Four, *Target sequence capture for phylogenomic research of diverse haemosporidian parasites infecting mammal and bird host species,* presents a novel malaria-UCE probe set and evaluates its utility using a sequence capture approach to obtain genomic data from avian and other related haemosporidian parasites. The sequence capture approach described here has promising potential in resolving complex evolutionary relationships, assessing population structure and testing phylogeographic hypothesis, that have not been possible yet, due to lack of genome-wide parasite data.

CHAPTER 2

TOWARDS A MORE HEALTHY CONSERVATION PARADIGM: INTEGRATING DISEASE AND MOLECULAR ECOLOGY TO AID BIOLOGICAL CONSERVATION [1]

[1] **Gupta, P.**, Robin, V. V., & Dharmarajan, G. (2020). Towards a more healthy conservation paradigm: integrating disease and molecular ecology to aid biological conservation. Accepted in *Journal of Genetics*. DOI: https://doi.org/10.1007/s12041-020-01225-7. Reprinted here with permission of publisher.

Abstract

Parasites, and the diseases they cause, are important from an ecological and evolutionary perspective because they can negatively affect host fitness and can be important in regulating host populations. Consequently, conservation biology has long recognized the vital role that parasites can play in the process of species endangerment and recovery. However, we are only beginning to understand how deeply parasites are embedded in ecological systems, and there is a growing recognition of the important ways in which parasites affect ecosystem structure and function. Thus, there is an urgent need to revisit how parasites are viewed from a conservation perspective and broaden the role that disease ecology plays in conservation-related research and outcomes. This review broadly focuses on role that disease ecology can play in biological conservation. Our review specifically emphasizes how the integration of tools and analytical approaches associated with both disease and molecular ecology can be leveraged to aid conservation biology. Our review first concentrates on disease-mediated extinctions and wildlife epidemics. We then focus on how elucidating host-parasite interactions has improved our understanding of the eco-evolutionary dynamics affecting hosts at the individual, population, community and ecosystem scales. We believe that the role of parasites as drivers and indicators of ecosystem health is an especially exciting area of research that has the potential to fundamentally alter our view of parasites and their role in biological conservation. The review concludes with a broad overview of the current and potential applications of modern genomic tools in disease ecology to aid biological conservation.

Keywords: conservation biology, ecosystem health, genetics, genomics, parasite, epidemiology, pathogen.

1. Introduction

Parasites are the most ubiquitous, but possibly one of the least understood, members of global ecosystems. Ranging from microscopic viruses to tapeworms measuring over 10 meters, parasites and the diseases they cause have long occupied a prominent place in social and scientific domains. However, despite our long fascination with parasites, we are only now beginning to understand how deeply they are embedded within ecological systems. This is especially important in present times when human-mediated alterations in the environment from global climate change to local habitat fragmentation – have led to dramatic declines in biodiversity, (Dirzo et al., 2014; Keith et al., 2015; Mace et al., 2012; Sato and Lindenmayer, 2018) and have altered the structure and function of natural ecosystems. Such anthropogenic alterations can interact in complex ways to directly or indirectly impact disease dynamics in human and wildlife populations (figure 2.1), creating a "geographic arena of pathogen emergence" (Hoberg and Brooks, 2015). Emerging and re-emerging diseases have been characterized as one of the most significant challenges of our times, and it is increasingly becoming clear that managing parasites in natural populations depends upon viewing disease as an eco-evolutionary process.

Most diseases in natural populations, including recently emerging or reemerging disease, are a complex interplay of multiple host and parasite species as well as the broader ecological community (including non-host species). This review focuses specifically on the role that parasites play in biological conservation, and comes a decade after the influential review by Smith et al. (2009) on the same topic. Consequently, here we primarily focus on the literature and/or concepts related to disease ecology that have developed in the past decade. Additionally, given the substantial growth in the fields of genetics and genomics, our review also focuses

specifically on how the integration of molecular and disease ecology can be leveraged to aid conservation biology. The first section focuses on the most apparent implications of parasites from a conservation perspective: disease-mediated extinctions and wildlife epidemics. The next sections take a step back and focus on how elucidating host-parasite interactions, specifically using genetic tools, has improved our understanding of the ecological and evolutionary dynamics affecting hosts at the individual, population, community and ecosystem scales (figure 2.1). We also discuss how parasites can serve as better ecological indicators at the host population and ecosystem scale. In the final section, we provide an overview of how recent advancements in molecular tools, especially high throughput DNA sequencing, have informed disease ecology. We believe that the role of parasites as drivers and indicators of ecosystem health is an especially exciting area of research that has the potential to fundamentally alter our view of parasites and their role in biological conservation.

2. Disease-mediated extinctions and epidemics in wildlife

There is an increasing recognition that wildlife diseases have important implications for biological conservation through their effects on biological diversity. Large-scale epidemics have occurred in natural populations leading to loss of biological diversity at local or global scales. Such epidemics have affected a wide variety of taxa, including: (1) Birds- One important group of emerging infectious diseases in birds are those attributed to avian haemosporidians (Apicomplexa: Haemosporida; *Plasmodium* and other related genera such as *Haemoproteus* and *Leucocytozoon*). Avian haemosporidians are a diverse group of vector-borne blood parasites that infect a wide array of bird taxa globally (Clark et al., 2014; Rivero and Gandon, 2018; Valkiŭnas, 2005). Large-scale mortalities in native wild birds have been well documented due to the accidental introduction of *Plasmodium* spp. and the vector *Culex quinquefaciatus* into island

bird communities which had no co-evolutionary history with these blood-borne parasites (e.g., Hawaii and New Zealand; Riper et al., 1986; Tompkins and Gleeson, 2006; Woodworth et al., 2005). (2) Amphibians- One of the most significant emerging infectious diseases affecting amphibians is caused by the chytrid fungus, *Batrachochytrium dendrobatidis*. Chytridiomycosis has been linked to population declines, local extirpations and even global extinctions (Pounds et al., 2006; Skerratt et al., 2007). Initial reports indicated that the fungus likely originated in Africa (Weldon et al., 2004), but a more recent genome-wide study suggests an Asian origin of *B. dendrobatidis*, and the pathogen was likely introduced globally with the expansion of commercial amphibian trade (O'Hanlon et al., 2018). Additionally, a newly discovered sister species, *B. salamandrivorans* has been associated with global population declines of salamanders (Martel et al., 2013; Yap et al., 2017). (3) Mammals- An important example of an emerging infectious disease-causing catastrophic declines in mammalian populations is the whitenose disease in bats. This disease is caused by the fungus *Pseudogymnoascus destructans*, a highly pathogenic fungus that colonizes the skin of hibernating bats (Blehert et al., 2009). The fungus was first identified in 2006 from New York and has caused local extirpations of many bats species, both endangered, (e.g., Indiana bat; *Myotis sodalist*; Thogmartin et al., 2013) and formerly common ones (e.g., little brown bat, *Myotis lucifugus*; Frick et al., 2010) across North America.

Apart from the diseases mentioned above, a number of other parasites have been implicated in populations declines including the rinderpest virus epidemic in wild ruminants in Kenya (Kock et al., 1999), the Ebola virus epidemic in Zaire that was responsible for the deaths of over 5000 gorillas (*Gorilla gorilla*; Bermejo et al., 2006), and the large-scale declines of North American bird populations due to West Nile virus (LaDeau et al., 2007).

In the face of large-scale epidemics, one major way in which molecular approaches can aid conservation is through the development of diagnostic tools to unambiguously identify parasites and quantify infection intensities. These approaches have already been leveraged to develop diagnostic tools for a wide range of parasites, including those that have been identified as being directly responsible for species endangerment, avian malaria parasites (Richard et al., 2002) and West Nile virus in birds (Kauffman et al., 2003), White-nose syndrome fungus in bats (Lorch et al., 2010) and the chytrid fungus in amphibians (Rosenblum et al., 2013). However, animal extinctions caused solely by disease are likely to be rare because they will only occur under a relatively restricted set of ecological conditions. First, when a parasite is a generalist, capable of infecting more than one species, it can persist in a reservoir host (i.e., a host that is relatively unaffected by the parasite; De Castro and Bolker, 2005). Indeed, most of the parasites cited above (i.e., *B. dendrobatidis*. *P. relictum*, *T. lewisi* and the Rinderpest virus) are generalist parasites infecting a broad diversity of hosts. Additionally, specific parasite strains may be more successful invaders into novel host communities compared to others (e.g. *P. relictum* invasion in New Zealand; (Ewen et al., 2012). The second mechanism that can lead to disease-mediated extinctions is frequency-dependent transmission (i.e., when transmission rate depends on the frequency of host contact, rather than host density; Boots and Sasaki, 2003). Classic examples of frequency dependent transmission include vector-borne diseases (e.g., West Nile virus and avian malaria), wherein high transmission rates can be maintained even at low host populations when the vector is efficient at finding their target species (Boots and Sasaki, 2003). Consequently, from a conservation perspective, the most important effects of parasites are likely to be driven by their effects on other eco-evolutionary factors that directly or indirectly affect the process of species endangerment and recovery (see details below). The rest of our review focuses on how

disease ecology, especially in conjunction with molecular ecology, can aid conservation by elucidating the varied eco-evolutionary interactions between parasites and their hosts.

3. Ecological and evolutionary dynamics

Parasites and the diseases they cause negatively affect organisms at multiple levels of biological organization: individuals, populations and species (Daszak et al., 2000; Lachish et al., 2011; Smith et al., 2009; Tompkins et al., 2011), and alter the structure and function of ecological communities. At the individual level, parasites directly impact survival: mortality of infected animals is 2.65 times higher than uninfected ones (see meta-analysis by Robar et al., 2010). Parasites can also reduce fitness through sub-lethal effects (e.g., impaired reproduction) or could reduce fitness through indirect mechanisms (e.g., increased predation risk; Hatcher et al., 2014; Seppälä and Jokela, 2008). Through their impacts on host fitness, parasites can also regulate host populations, limit host abundance, and lead to large-scale epidemic mortalities (see above). Furthermore, parasites can alter the outcome of species interactions (e.g., competition, predation) and influence population and community structure. Finally, it is important to recognize that parasites are deeply embedded within our ecosystems and the effects of parasites can cascade through all trophic level via alteration of food web structure, flow of energy and nutrients (Hatcher et al., 2012a; Wood and Johnson, 2015).

3.1. Parasite transmission dynamics at the individual level

Numerous factors acting at the scale of the individual can impact parasite transmission dynamics. Here we focus on two factors, host genetic diversity and anti-parasite defense, that modify host susceptibility to infection.

Host genetic diversity

Parasites constitute a robust selective force on host populations because of their rapid evolutionary rate that can promote the maintenance of host genetic diversity (Ladle, 1992). Many studies have indicated that host genetic diversity plays an important role in buffering host populations from diseases (King and Lively, 2012). However, the relative importance of neutral vs. adaptive genetic diversity has long been questioned in the field of conservation biology (e.g., Hughes 1991; Vrijenhoek and Leberg 1991). Low levels of neutral genetic diversity or high levels of inbreeding in natural populations are expected to increase host susceptibility to disease due to reduced adaptive potential. For instance, genetic diversity at neutral loci (measured as marker-based heterozygosity) has been shown to be negatively associated with susceptibility to parasites in insects (e.g. Whitehorn et al., 2011), birds (e.g., Luikart et al., 2008; Ortego et al., 2007a; Townsend et al., 2018), mammals (e.g., Mitchell et al., 2017; Rijks et al., 2008) and fish (e.g., Eszterbauer et al., 2015; Hedrick et al., 2001; Smallbone et al., 2016). Empirical evidence supports the perspective that inbred individuals generally tend to be more susceptible to parasites compared to outbred ones (Hedrick et al., 2001). Inbreeding has also been associated with increased disease severity. For example, American crows (*Corvus brachyrhynchos*) that died with disease symptoms associated with West Nile Virus infections showed higher levels of inbreeding as compared to birds that died of other causes (Townsend et al., 2009). Similarly, European treefrogs (*Hyla arborea*) from inbred populations died more quickly when exposed to *B. dendrobatidis* compared to those from outbred populations (Luquet et al., 2012).

However, the association between genetic diversity and parasitism is not universal, with some studies finding no association (Côté et al., 2005; Ortego et al., 2007b), or variable effects based on the strength of parasite-mediated selective pressures (Ruiz-López et al., 2012).

Additionally, since inbreeding is negatively correlated with genetic diversity (e.g., marker-based heterozygosity), it is generally assumed that these metrics will affect parasite infection in opposite directions. Interestingly, Mitchell et al. (2017) found that while increased heterozygosity was associated with lower parasite loads in wild banded mongooses (*Mungos mungo*), there was no association with the pedigree-based inbreeding coefficient, likely due to linkage with genes influencing parasite burdens. Importantly, as genetic diversity increases there is also a concomitant increase in the prevalence of rare genotypes in the population. The rarity of a particular host genotype could affect infection risk if parasites tend to be co-adapted to more common genotypes, as predicted by the Red Queen hypothesis (Decaestecker et al., 2007; Lively et al., 1990; Lively and Dybdahl, 2000). However, few studies have explicitly tested the relative effects of genotype rarity and heterozygosity on infection risk in wildlife. In an elegant study, Eastwood et al. (2017) show that both heterozygosity and genotype rarity affect Beak and Feather Disease Virus infection risk in wild parrots (*Platycercus elegans*). Importantly, these authors show that heterozygosity reduced infection risk (but not infection load), while host genotype rarity decreased viral loads in infected individuals (but did not affect infection risk).

In contrast to neutral loci, functionally important loci (e.g. immune loci) can be more informative because genes associated with immune function generally seem to evolve more rapidly than other areas of the genome (Lazzaro and Clark, 2012; Nielsen et al., 2005; Shultz and Sackton, 2019). The major histocompatibility complex (MHC) is one of the most studied immune gene families in wild vertebrates due to its high variability and central role in adaptive immune response (Acevedo-Whitehouse and Cunningham, 2006). Numerous studies have reported a negative relationship between MHC diversity and parasitism risk or disease severity in mammals (Caldwell and Siddle, 2017; Sin et al., 2014), reptiles (Elbers and Taylor, 2016),

amphibians (Fu and Waldman, 2017), and fish (Wegner et al., 2003). However, these patterns are not universal, with no effects of MHC diversity on infection risk in New Zealand passerine birds (Sutton et al., 2016) or giant pandas (*Ailuropoda melanoleuca;* Zhang et al. 2015).

MHC diversity could have important implications for community-level processes (e.g., interspecific competition). For example, the decline of red squirrel (*Sciurus vulgaris*) in the UK is likely due to apparent competition with the invasive eastern grey squirrel (*S. carolinensis*) mediated by squirrelpox disease (see details in section on *Biological Invasions*). Importantly, recent data have indicated that the increased susceptibility of red squirrels to the disease may be driven by MHC diversity because red squirrel populations in the UK have much lower MHC diversity compared to populations in continental Europe (Ballingall et al., 2016). Several studies have also revealed that selection tends to maintain high MHC diversity even in populations that have undergone recent, severe bottlenecks and have dramatically reduced neutral genetic diversity in numerous species including: Hume's pheasant, *Syrmaticus humiae* (Chen et al., 2015), zebra finches, *Taeniopygia guttata* (Newhouse and Balakrishnan, 2015), The lowland leopard frog, *Lithobates yavapaiensis* (Savage and Zamudio, 2016), the alpine ibex, *Capra ibex* (Brambilla et al., 2018), black-spotted pond frog, *Pelophylax nigromaculatus* (Wang et al., 2017) and European rabbits, *Oryctolagus cuniculus* (Schwensow et al., 2017). Strong balancing selection on MHC loci has also been shown in some populations, thus maintaining relatively uniform MHC diversities, despite significant population genetic differentiation at neutral markers (Niskanen et al., 2014). However, this study also revealed that in some parasites, individual MHC alleles may be more important than overall MHC diversity (Niskanen et al., 2014). Other studies have found similar patterns. For example, particular conformations of the MHC class II PBR appear to confer resistance to the chytrid fungus and these alleles are shared

by parasite-resistant amphibian species globally (Fu and Waldman, 2017). Similarly, in the case of avian malaria, individual MHC alleles or supertypes (functional clustering of alleles) seem to confer resistance to parasite infection in a wild great tit, *Parus major,* population (Sepil et al., 2013).

While MHC has been well studied, factors affecting MHC diversity in natural populations could be complex. For example, it has been hypothesized that mate choice should tend to maximize MHC diversity in offspring to improve resistance to parasites (Von Schantz et al., 1996) and survival (Agbali et al., 2010). However, a meta-analysis across a wide variety of taxa suggests that the direct effects of sexual selection may outweigh those associated with parasites in maintaining MHC variation (Winternitz et al., 2013), and there is evidence that both diversity- and dissimilarity-based mate choice act to maintain MHC diversity (Kamiya et al., 2014).

Another critical issue is that much of our understanding of wildlife immunogenetics has primarily focused on the MHC family of genes, and there is a need to expand the immune genes studied in wildlife. Many such candidate genes exist including chemokine, interleukin and Toll-like receptors, and interferon and tumor necrosis factor genes (Acevedo-Whitehouse and Cunningham, 2006). Some of these genes have been investigated for associations with disease or immune competence in wildlife. For instance, Turner et al. (2012) reported that parasite-influenced selection maintains genetic diversity in cytokines, genes critical for initiating and mediating the immune response, in field voles (*Microtus agrestis*). A large-scale capture-mark-recapture study of wild bank voles (*Myodes glareolus*) showed that individual infection risk for *Borrelia* spp. infection was associated with specific genetic variants at the Toll-like receptor gene (Tschirren et al., 2013). Additionally, a study by le Roex et al. (2013) revealed that three

SNPs located in genes with predicted immune function were associated with bovine tuberculosis infection in the African buffalo (*Syncerus caffer*). Many studies in cervids also have shown that amino acid polymorphisms in the prion protein gene affect resistance to chronic wasting disease (Brandt et al., 2018; Cheng et al., 2017; Monello et al., 2017). Finally, in one of the most comprehensive studies to date, Bateson et al. (2016) showed that in the endangered Attwater's prairie-chicken (*Tympanuchus cupido attwateri*), innate (Toll-like receptors, TLRs) and adaptive (major histocompatibility complex, MHC) immune systems, but not genome-wide heterozygosity, affected the post-release survival of captive-bred birds.

Anti-parasite defense

Traditional models of parasite virulence generally assume that parasites cause damage to their hosts because they acquire resources from their hosts to reproduce, and thus transmit infections to new hosts (Frank, 1996). Thus, host-parasite interactions have traditionally been viewed as being antagonistic, with hosts maximizing fitness primarily by negatively impacting the parasite's fitness through the development of resistance mechanisms. Such mechanisms either act to reduce infection risk (e.g., through behavioral mechanisms) or to reduce parasite burden once infected (e.g., immune-mediated killing; De Roode and Lefèvre, 2012; Medzhitov et al., 2012; Råberg et al., 2009; Soares et al., 2017). However, it is increasingly being recognized that hosts can also minimize harm (fitness cost) inflicted at a given parasite burden through tolerance mechanisms (e.g., tissue repair; Ayres and Schneider, 2012; Kutzer and Armitage, 2016; Råberg et al., 2009, 2007; Schneider and Ayres, 2008; Soares et al., 2017). The importance of parasite tolerance in plants has long been recognized (Caldwell et al., 1958; Pagán and García-Arenal, 2018), but only recently, have animal ecologists started investigating the role tolerance plays in shaping host-parasite interactions (above). Pioneering studies have revealed that

variation in tolerance in insects could ameliorate infection-mediated reduction in survival (Ayres and Schneider, 2009; Vincent and Sharp, 2014) or fecundity (Parker et al., 2014).

While both resistance and tolerance improve host fitness in the face of parasite attack, they are expected to have very different outcomes. From an epidemiological perspective, resistance is expected to reduce parasite prevalence and select for increased parasite virulence, while tolerance will have neutral or even positive effects on both prevalence and virulence (Ayres and Schneider, 2012; Råberg et al., 2009; Schneider and Ayres, 2008). From an evolutionary perspective, since tolerance does not directly reduce parasite fitness, antagonistic coevolution (i.e., Red Queen dynamics) is not expected in the case of tolerance, unlike in the case of resistance (Ayres and Schneider, 2012; Kutzer and Armitage, 2016; Råberg et al., 2009; Schneider and Ayres, 2008).

The relative investment in resistance vs tolerance can indeed affect disease dynamics at multiple scales of biological organization. For example, highly tolerant individuals are expected to be efficient at transmitting disease in a population (i.e., super-spreaders; Gopinath et al., 2014). Additionally, species that are tolerant to parasite infection may serve as reservoirs of infection and amplify the risk for vulnerable species. For example, the introduction of grey squirrels (*Sciurus carolinensis*) to Great Britain led to a decline of the native red squirrel (*Sciurus vulgaris*) populations as grey squirrels were carriers of Parapoxvirus and were tolerant to infection (Rushton et al., 2000; Tompkins et al., 2002). Introduction of avian malaria in the Hawaiian Islands caused mass mortality and extinction of several native forest birds due to a virulent strain of *Plasmodium relictum* (GRW04) (Atkinson et al., 2000; Warner, 1968). Empirical evidence suggests that lowland populations of Hawaii Amakihi (*Hemignathus virens*) that survived the infection had evolved tolerance as a defense strategy against avian malaria and

may serve as reservoirs of local parasite populations (Atkinson et al., 2013). Vector-borne disease dynamics can also be affected by the evolution of tolerance in the vector. A recent study quantifying parasite resistance and tolerance in natural mosquito populations revealed that mosquitoes colonized from high transmission intensity areas had higher tolerance and lower resistance, as compared to those colonized from low transmission intensity (Dharmarajan et al., 2019). Further, as tolerance was associated with increased vectorial capacity (i.e., the probability of a mosquito transmitting the parasite), this study showed that transmission intensity can impact vector evolution, which can in turn feedback to impact disease risk.

The view of parasitic infections from a resistance-tolerance perspective necessarily delinks parasite infection risk and parasite burden from fitness consequences. For example, highly tolerant populations could have high parasite burdens with low fitness costs. Alternatively, populations with high resistance may have low parasite burdens but face costs associated with immunopathology. Consequently, the importance of eco-immunology (Hawley and Altizer, 2011) and ecophysiology (Blaustein et al., 2012) both contribute to the progression of infection to disease. Methodological approaches to the measurement and analysis of resistance and tolerance in parasite populations are possible and continue to grow. However, there are multiple challenges in the interpretation of parasite load (e.g., the relative effects of increased infection risk vs. reduced resistance), and the implications of parasite load on fitness (e.g., the confounding effects of immune- vs. parasite-mediated pathology). Additionally, quantifying fitness and individual health in natural populations is a challenging issue. For instance, body-condition indices, a commonly used surrogate measure of fitness in ecological studies have been associated with some fundamental issues (e.g., many animals maintain body composition at homeostasis; Wilder et al., 2016). A recent meta-analysis reveals that while there is evidence for

a negative relationship between condition indices and infection risk, the magnitude of this effect is relatively small and that there is evidence for publication bias towards negative relationships (Sánchez et al., 2018).

3.2. Parasite transmission dynamics at the population level

Numerous mechanisms acting at the population scale can affect parasite transmission dynamics. Here, we focus on two of the most important factors, host spatial and social structure, that can influence heterogeneity in contact rates, and thus parasite transmission risk.

Spatial structure

Molecular tools in conjunction with population/landscape genetic and phylogeographic analyses have been used to elucidate ecological and evolutionary processes at multiple scales of biological organization (Manel and Holderegger, 2013; Petren, 2013; Robin et al., 2015b). The integration of such genetic methods with epidemiological information (e.g., parasite prevalence) has provided critical insights into disease transmission dynamics and epidemiological history of emerging infections (Archie et al., 2009). Levels of geneflow can help characterize the spatial spread of phenotypes of epidemiological importance (e.g., virulence, drug and pesticide resistance) in parasite and vector populations, and also help quantify parasite invasion risk (Schwabl et al., 2017). Thus, genetic data can aid biological conservation by providing insights into how ecological factors affect the functional connectivity of parasites, hosts and vectors at the individual or population scales. For example, fine-scale genetic data has been used to elucidate the spatio-temporal factors affecting how individual wildlife hosts sampled ticks from their environment (Dharmarajan et al., 2011, 2010). Specifically, this research produced empirical evidence for the aggregated transmission of parasites as “packets” of related individuals, and such transmission dynamics, along with a subdivided mating system (i.e. mating

at the host scale), could explain the deviations Hardy-Weinberg Equilibrium expectations which is common in many parasites (Dharmarajan et al. 2011). Critically, such aggregated transmission dynamics in conjunction with host spatial structure could have critical epidemiological implications (e.g., the spread of recessive drug resistance genes; Dharmarajan 2015).

Underlying landscape features can critically alter host movement patterns and consequently influence the spatial spread of parasites (Kozakiewicz et al., 2018). Landscape genetics has been an extraordinarily powerful approach that has been used effectively to identify landscape features influencing the spatial spread of parasites, including chronic wasting disease prions due to movement of deer (Brandt et al., 2018; Cullingham et al., 2011b, 2011a; Kelly et al., 2014) and rabies virus due to movement of hosts like raccoons (*Procyon lotor*) and skunks (*Mephitis mephitis*) (Biek et al., 2006; Biek and Real, 2010; Heloise et al., 2012). However, landscape features do not necessarily limit animal movement in all species and may facilitate parasite spread. For example, Deyoung et al. (2009) were unable to identify natural boundaries to geneflow, and thus rabies spread, for gray foxes (*Urocyon cinereoargenteus*). Landscape genetics approaches also form a powerful approach to quantify dispersal resistance/permeability surfaces for natural populations. For example, Bouyer et al. (2015) used microsatellite data to develop landscape resistance surfaces and thus identify candidate populations for Tsetse fly (*Glossina palpalis gambiensis*) elimination.

Alternatively, the genetic structure of parasite populations can be used to elucidate characteristics of the host population that could not be obtained from host genetics alone (Dharmarajan et al., 2016). In the case of migratory birds, host genetics is a poor indicator of migratory routes because genetic recombination only occurs in the breeding (summer) but not in non-breeding (winter) grounds. However, critical insights into migratory strategies can be

obtained through the genomic characterization of parasites, such as avian influenza virus in waterfowl (Hill et al., 2012b, 2012a; Newman et al., 2012).

Using phylogenetic approaches to analyze high-resolution parasite genotype data can help detect and date historical changes in epidemiological patterns (Stadler and Bonhoeffer, 2013), which can have implications for conservation. For example, genome resequencing of the chytrid fungus, shows that fungus evolved in East Asia, and that the evolutionary history of this parasite likely predates its continent-scale movement (Rosenblum et al., 2013), which has likely been driven by commercial trade in amphibians (Fisher et al., 2012; James et al., 2009; O'Hanlon et al., 2018). Similarly, sequencing of the hemagglutinin gene of canine distemper virus isolates from over a period of four decades revealed that the virus emerged in the United States in the late 1800s, with subsequent diversification and global spread, likely due to uncontrolled animal trade and human transfer of pets (Panzera et al., 2015). Spatial spread of parasites is not necessarily associated with phylogenetic divergence. For example, in the case of Yersinia species, whole-genome sequencing has revealed a deep phylogenetic split between pathogenic strains and nonpathogenic lineages. However, all pathogenic Yersinia species do not share a recent common pathogenic ancestor, but instead they seem to have converged independently to acquire the same virulence determinants (Reuter et al., 2014).

For parasites that evolve rapidly, phylodynamics – an analytical method combining phylogenetics, epidemiology, population genetics, and immunology (Baele et al., 2017; Grenfell et al., 2004; Leventhal et al., 2012) – is a powerful approach that has been used to elucidate ecological (e.g., transmission bottlenecks; Volz et al., 2017) and evolutionary (virulence heritability; Vrancken et al., 2015) dynamics. RNA viruses are particularly well suited as markers of host ecology because they are spread via direct contact and have higher mutation

rates compared to the host genome (Volz et al., 2013). Because of their relatively rapid evolutionary rate, viruses can reveal patterns of host movement and provide insight into patterns of disease distribution and spread that may not be apparent in the host genetic data, and thus provide a complementary tool for studying population dynamics of their hosts in "shallow" time. For example, genetic patterns associated with Feline Immunodeficiency Virus isolates from mountain lions (*Puma concolor*) in the Rocky Mountains of North America revealed pronounced spatial genetic structure providing information on the recent demographic history that was not evident from the host microsatellite data (Biek et al., 2006).

Social structure

Many animals show spatial heterogeneity in contact networks due to underlying ecological or environmental factors affecting levels of social structure. Social groups size is generally driven by levels of competition for critical resources such as food (e.g., in jackals, *Canis aureus*; MacDonald 1979) or mates (e.g., in the striped mouse, *Rhabdomys pumilio*; Schradin et al., 2012). Aggregation of resources, such as supplemental feeding, can increase infectious disease transmission risk (Becker et al., 2015; Brook et al., 2012; Sorensen et al., 2013). Thus, the clustering of birds around bird feeders during winter has been linked to mycoplasmal conjunctivitis outbreaks in North America (Dhondt et al., 2005). There is generally a positive association between group size and parasite infection risk (Griffin and Nunn, 2012), and thus the elevated risk of parasitism could be a substantial cost associated with sociality. For example, gorillas (*Gorilla gorilla*) in Central Africa that clustered around seasonally fruiting trees were more affected by the outbreak of Ebola (97% mortality) compared to solitary individuals (77% mortality; Caillaud et al. 2006). At the population level, parasite transmission rates are expected to be higher in populations with low levels of social or spatial structure (i.e.,

smaller group sizes and more movements between groups; Craft 2015). For example, in the case of lions (*Panthera leo*), infection risk in animals living in prides is epidemiologically similar to homogeneous populations up to 20 percent larger (Caillaud et al., 2013).

Spatial aggregation of related individuals (i.e. kin structure) is a common form of social organization in wildlife (Altizer et al., 2003). Generally, we would expect increased levels of social interactions between related vs. non-related individuals, and several studies have confirmed this pattern using epidemiological data. For instance, higher contact rates within family groups have been demonstrated in white-tailed deer (*Odocoileus virginianus*) because deer infected by bovine tuberculosis (a parasite transmitted primarily by direct contact) are more closely related than non-infected deer (Blanchong et al., 2007). Similar findings have been reported in the case of chronic wasting disease in deer (Cullingham et al., 2011a; Magle et al., 2013). However, the effects of host relatedness on disease risk, will depend upon the spatial scale relevant for parasite transmission. For example, in raccoons infection risk associated with a directly transmitted parasite (canine distemper) was positively related to contact rates (i.e., familial structure) within spatially discrete habitat patches, while infection risk associated with an environmentally transmitted parasite (*Leptospira* spp.) was positively related to contact rates (i.e., geneflow) amongst the patches (Dharmarajan et al., 2012).

Social networks critically affect parasite transmission dynamics in wildlife (White et al., 2017), and consequently parasite networks can be used to provide valuable, and often unique, insights into contact/association patterns in natural populations. For example, in an interesting study, Chiyo et al. (2014) found that bacterial networks were surprisingly not associated with social structure in African elephants (*Loxodanta africana*), but rather driven primarily by habitat utilization patterns or individual host characteristics (i.e., sex and age). However, other studies

have found that bacterial transmission networks strongly reflect underlying social structure of their hosts including sleepy lizards (*Tiliqua rugosa*; Bull et al., 2012) and giraffes (*Giraffa camelopardalis*; VanderWaal et al., 2014).

3.3. Parasite transmission dynamics at the community and ecosystem level

While disease ecology has traditionally focused on dynamics at the individual and population scale, there is increasing evidence that community and ecosystem level process also critically affect transmission dynamics.

Species interactions

As in the case of individual species (see above), bacterial transmission networks have also been used to characterize patterns of species interactions in multi-species communities. For example, genetic analysis of *Escherichia coli* across ten species of wild and domestic ungulates in Kenya revealed that the Grant's gazelle (*Gazella granti*) showed the highest number of network connections in the community. However, the zebra (*Equus burchelli*), which tends to move longer distances than many other ungulates in this system, connected regions of the network that would otherwise have been poorly connected (VanderWaal et al., 2014b). Such studies at the community level have especially significant ramifications for emerging infectious diseases because characterizing disease emergence often entails a better understanding of when a parasite will spill over from one host species into another.

Ecological filters critically affect disease emergence because host switching is more likely for sympatric host species, and many emerging viruses in humans have zoonotic origins (e.g., SARS, corona and hanta viruses; Jones et al., 2008a). Consequently, characterization of viruses harbored by potential wildlife reservoirs is a potential tool to predict or prevent disease emergence. For example, a metagenomic survey of viruses in the urine of the Old World fruit bat

(*Eidolon helvum*) revealed that bats roosting close to humans harbored a wide variety of viruses, some of which are genetically related to known human parasites, highlighting the risk of zoonotic transmission (Baker et al., 2013). Whole genome sequence data has also been used to characterize the parasite transmission at the wildlife-domestic animal interface, including the transmission of bovine tuberculosis from badgers (*Meles meles*) to cattle (Biek et al., 2012) and the spatial spread of *Brucella abortus* among livestock, bison (*Bison bison*) and elk (*Cervus canadensis;* Kamath et al., 2016).

Another novel way in which genetic tools (e.g., sequencing of mitochondrial genes) have been used to elucidate the potential for interspecific disease transmission is through the analyses of blood meals in disease-transmitting vectors such as mosquitoes (Crabtree et al., 2013; Lutomiah et al., 2014), blackflies (Hellgren et al., 2008) and ticks (Gariepy et al., 2012). Such studies can be crucial for transmission dynamics. For instance, mosquito blood meal analysis combined with knowledge of host competence was used to identify which bird species were most important in the amplification of West Nile virus (Hamer et al., 2009). This study predicted that the virus was primarily maintained by just three bird species (American robins, *Turdus migratorius*; blue jays, *Cyanocitta cristata*; house finches, *Carpodacus mexicanus*) which constituted a large proportion (35%, 17% and 15%, respectively) of hosts fed on by West Nile virus-infectious *Cx. pipiens*. From a conservation perspective, a particularly exciting avenue is that vector blood meal analyses can be used as a tool for biodiversity prospecting (Bohmann et al., 2013; Lee et al., 2015; Schnell et al., 2015).

Biodiversity-disease relationship

The importance of host community structure on disease risk, especially zoonotic disease risk, in humans has been an area that has been especially contentious (Civitello et al., 2015). The

dilution effect hypothesis posits host communities with high diversity are comprised of more hosts that are either refractory or less susceptible to parasites, and that such hosts “dilute” the risk of disease in more competent hosts (Johnson et al., 2015b; Ostfeld and Keesing, 2012). Consequently, this hypothesis predicts that reduced disease risk could be an “ecosystem service” associated with biodiversity (LoGiudice et al., 2003; Wood and Johnson, 2015). Other authors have argued that the "biodiversity protects against disease" paradigm is Panglossian because it provides a questionable utilitarian justification for protecting biodiversity, and that the specific composition of the host community critically decides whether biodiversity dilutes or amplifies disease risk (Randolph and Dobson, 2012). The concept behind the dilution effect is not in itself new, and this has been proposed earlier in the ecological literature (e.g., the “decoy effect”; Chernin, 1968), or has been effectively used to control vector-borne disease (e.g., zooprophylaxis; Hess and Hayes, 1970). A recent meta-analysis has provided strong support for the dilution effect independent of host density, study design, and type and specialization of parasites (Civitello et al., 2015). However, there remains no definitive conclusion on the dilution effect debate (e.g., Ostfeld, 2013; Randolph and Dobson, 2012), and as in most ecological debates the answer likely lies “somewhere in the middle”. For example, Keesing and Ostfeld, (2012) mention that in two of the best case studies of the dilution effect (Lyme disease and West Nile virus), the most competent host for the parasite (white-footed mice, *Peromyscus leucopus* and American robins, *Turdus migratorius*, respectively), are also the most resilient hosts that reach high abundance in degraded habitats. Basic ecological niche theory predicts that increasing levels of species diversity will favor the evolution of narrower niches to minimize niche overlap between species in a community. Consequently, generalist species are expected to dominate ecological communities with low species diversity and vice versa. Thus the universality of the

dilution effect could be driven by two potential mechanisms (Keesing and Ostfeld, 2012): successful parasites are those that have adapted to generalist hosts or that generalist hosts have evolved life history strategies that minimize allocation of resources to particular types of anti-parasite defense mechanisms.

Parasites and biological invasions

Invasive species are pervasive biotic agents that can impact native community structure and threaten global biodiversity, and the dual role of parasites as drivers of, as well as passengers responding to, altered community structure is especially important from the perspective of biological invasions (Blackburn and Ewen, 2017; Dunn et al., 2012; Prenter et al., 2004). Disease is as an important driver or consequence associated with nearly a quarter of species listed in the IUCN's list of the world's worst invaders (Hatcher et al., 2012b), and models reveal that parasites can affect the magnitude and rate of invasive species (Coates et al., 2017; Hilker et al., 2005).

Parasites can drive the success (or failure) of biological invasions through three mechanisms: (1) Enemy Release Hypothesis (Keane and Crawley, 2002) proposes that some invasive species may escape the adverse fitness effects (e.g., on survival or reproduction) associated with parasites in their native range and thrive better in their introduced range. This effect of parasites has been compared to the kryptonite effect (Hudson et al., 2006) because Superman gained super-powers on Earth because he was freed from the effects of kryptonite. A meta-analysis found that parasite diversity and prevalence were higher in native vs. exotic populations (Torchin et al., 2003), indicating that enemy release could be common in biological invasions; For example, the European green crabs that were introduced in North America carried only a subset of parasites from their native range, and consequently were larger in size and had better reproductive success; (2) Novel Weapon Hypothesis (Price et al., 1986): proposes that

parasites can be co-introduced with invasive species that may spill over to native species. This can negatively impact the native species by influencing the competitive interactions between invasive and native species (i.e., through apparent- or parasite-mediated competition). For instance, as mentioned earlier, the introduction of grey squirrels to Great Britain, which introduced paprapox virus and replaced native red squirrels is one of the best-documented cases of such parasite-mediated invasion. The paprapox virus caused a 20 fold greater mortality in red vs. gray squirrels (Rushton et al., 2000; Tompkins et al., 2003); (3) Biotic Resistance Hypothesis (Elton, 1958), is similar but opposite to the Novel Weapons hypothesis, and proposes that introduced species could be negatively affected by resident parasite communities. Thus, generalist parasites could directly affect ecosystem stability through increased virulence in non-adapted alien hosts (i.e., Suicide King Dynamics). However, more broadly, it has been recognized that ecological diversity and complexity help maintain ecosystem resilience, and parasites may be important components of ecosystems because they constitute a large proportion of species diversity and dominate food web links in most ecosystems (see below). Consequently, it has been proposed that a healthy ecosystem is one that has high parasite species richness (Hudson et al., 2006).

While parasites may drive biological invasions, it is also possible that they are just passengers associated with biological invasions. The co-introduction of parasites with invasive species seems to be more common than previously recognized (Lymbery et al., 2014). Such co-introduced parasites are not only powerful tools for the reconstruction of invasion history (e.g., Blakeslee and Byers, 2008), but may also alter the outcome of invasion (Lymbery et al., 2014). For example, a meta-analysis shows that parasite spillover from native to alien species is common, with native parasite species accounting for over 60% of the parasite fauna across a

wide range of invasive taxa (Kelly et al., 2009). However, these authors also show that non-indigenous species can be highly competent hosts for such parasites and that there is evidence for spillback of these native parasites from the invasive to native species (Kelly et al., 2009). Consequently, parasites can affect and be affected by the structure of their host communities.

Parasites and ecosystem health

Myriad human-mediated environmental perturbations – habitat modification, chemical contamination and global climate change – increasingly are impacting ecosystem structure and function, and impairing the resilience of these systems to such perturbations. One way in which environmental perturbations affect human/wildlife populations is through altered disease dynamics, and consequently the emergence of novel pathogens or re-emergence of old ones (Rogalski et al., 2017). Such epidemiological endpoints are critical from the perspective of ecosystem health for three reasons. First, the sensitivity of disease dynamics to environmental modification makes parasites one of the best indicators of ecosystem health (see below). Second, altered disease dynamics can feedback on ecosystem health through its impact on various processes from community composition to nutrient cycling (Horwitz and Wilcox, 2005; Johnson et al., 2015a; Preston et al., 2016). Third, the link between the altered structure and function of ecological systems and the health of humans, domestic animals and wildlife at individual and population scales is well recognized (Cable et al., 2017; Rogalski et al., 2017; figure 2.1), and these concepts have particular importance from the perspective of emerging infectious disease (Bird and Mazet, 2018). In this section we primarily focus on how parasites form an integral component of ecosystems and can alter food web structure, flow of energy and nutrients, and drive trophic cascades.

Parasites play an important role in maintaining the structure and functioning of food webs since they constitute a large proportion of total biomass in many ecosystems (Kuris et al., 2008; Preston et al., 2013). In one of the first empirical studies, Kuris et al. (2008) estimated that parasite biomass was similar to fishes and many invertebrate taxa; and exceeded that of avian predators in estuarine ecosystems. Similarly, it has been shown that the annual biomass of trematode larval stages could be up to ten-fold more than winter bird biomass and exceeded that of most aquatic invertebrate taxa (Preston et al., 2013). Besides their contribution to productivity in ecosystems, parasites can increase food web complexity by increasing connectivity, chain length and nestedness. For instance, studies have shown that parasites comprise a large proportion of trophic links (ranging from 29-78%) in aquatic ecosystems (Amundsen et al., 2009; Hernandez and Sukhdeo, 2008; Lafferty et al., 2006; Preston et al., 2014). The centrality of parasites in ecological networks is not surprising because, from a trophic perspective, parasites are predators, and they can thus have cascading effects on many essential ecosystem processes, such as decomposition (Sato et al., 2011) and grazing (Cleaveland et al., 2009). However, unlike true predators, parasites can also transfer energy within trophic levels (e.g., direct transmission of parasites between herbivores through grazing). Additionally, energy can move from parasites to true predators through direct (e.g., consumption of infected prey or parasite free-living stages; Johnson et al., 2010) or indirect (e.g., increasing susceptibility of intermediate hosts to predation through behavioral modification; Lefèvre et al., 2009) mechanisms.

Parasites, by definition, survive and reproduce by using resources obtained from their hosts. However, surprisingly few empirical studies have highlighted the importance of parasites for cycling nutrients at the ecosystem scale (Vannatta and Minchella, 2018). For example, frog

tadpoles are dominant grazers in many stream ecosystems, and consequently high (98%) mortality of tadpoles due to chytrid fungus infections were associated with strong trophic cascades (increased algae and fine detritus biomass) and a 50% reduction in nitrogen cycling (Whiles et al., 2012). The study by Connelly et al. (2008) showed that chytrid-mediated local extirpation of over 90% of tadpoles led to similar large-scale trophic cascades and altered nutrient cycling in natural streams, including a 269% increase in chlorophyll, 220% increase in ash-free dry mass and 140% increase in inorganic sediments. Fungus-mediated mortality in alder trees (*Alnus tenuifolia*) has also been shown to impact nitrogen cycling of flood plain ecosystems (Ruess et al., 2009). Other studies have found that parasite-mediated effects on nutrient cycling dynamics are similar/larger in magnitude than those attributed to better-characterized disturbances, such as climate change and air pollution (Lovett et al., 2010) or forest fires (Cobb et al., 2012).

As may be expected in the case of true predators, parasites can also drive trophic cascades by decreasing the abundance of herbivores and releasing producers from the pressures of herbivory (reviewed by Buck and Ripple, 2017). For instance, parasite-mediated mortality of rabbits due to Myxoma virus (Dobson and Crawley, 1994), large ungulates due to Rinderpest virus (Cleaveland et al., 2009), and amphibians due to chytrid fungus (Connelly et al., 2008; Whiles et al., 2012) have all led to cascading effects on producers. There are also examples wherein parasite-mediated mortality of true predators was associated with the release of herbivores from predatory pressures, including the mortality of red foxes (*Vulpes vulpes*) due to ectoparasitic mites (Lindström et al., 1994), and wolves (*Canis lupus*) due to canine parvovirus (Wilmers et al., 2006). Alternatively, because parasites are critically affected by host community structure, trophic cascades can feedback to alter disease dynamics. For example, long-term

exclusion of herbivores has been associated with altered disease dynamics in grassland ecosystems (Dirzo et al., 2014). A recent study, (Weinstein et al., 2017) shows that the removal of large herbivores leads to increased rodent densities (due to reduced competition), which in turn leads to an increase in rodent-borne parasites. Consequently, parasites can be "drivers" of trophic cascades or can simply be "passengers" that respond to trophic cascades.

Parasites can serve as excellent indicators of ecosystem health. Historically, coal miners used canaries to detect low levels of carbon monoxide and methane gas (Holt and Miller, 2011), and it has been proposed that parasites can act as "canaries" to detect alterations in the health of ecological systems. One way in which parasites have been effectively used as bio-indicators is with respect to accumulation of environmental contaminants, which may be driven by their higher trophic position as compared to their hosts. For example, concentrations of heavy metals in the tissue of certain parasites, like acanthocephalans of fish (Nachev and Sures, 2016; Sures, 2001; Sures et al., 2017b) and pinnipeds (McGrew et al., 2018) can be several magnitudes higher than those present in host tissues or environment. However, this pattern is not universally the case. For example, Tellez and Merchant (2015) found that while parasites (i.e., intestinal trematodes) showed higher concentrations of some heavy metals (e.g., arsenic and zinc), hosts (i.e., the American alligator, *Alligator mississippiensis*) were better indicators of others (e.g., cadmium and lead).

Parasites may also be effective indicators of ecosystem structure and function because they likely are sensitive to perturbations, including anthropogenic effects on ecological systems, that affect host community structure, species interaction dynamics and food web topology (Marcogliese, 2005). One area that has received a lot of attention is in testing the effects of specific anthropogenic modifications of the environment, such as pollution or land-use change

(e.g., urbanization) on parasite infection risk or community structure. With respect to parasite infection risk (i.e., parasite loads), despite several decades of effort, there remain no general trends, and three influential meta-analysis have found that effects of contaminants on parasite loads could be positive, negative, or absent depending on the parasite and pollutant (Blanar et al., 2009; Lafferty, 1997; Sures, 2008; Vidal-Martínez et al., 2010). Alternatively, contaminants seem to have a relatively consistent and significant negative effect on species richness (Blanar et al., 2009). For example, Calegaro-Marques and Amato (2014) found that helminth species richness in rufous-bellied thrushes (*Turdus rufiventris*) was inversely related to the degree of urbanization, and hypothesized that structures in urbanized landscapes (i.e., buildings) act to break up host-parasite interactions. Similarly, *Toxoplasma gondii* genotype diversity was found to decrease in areas of human settlement (Jiang et al., 2018), and lead intoxication was found to reduce helminth species richness in mallards (*Anas platyrhynchos*; Prüter et al., 2018). It has also been proposed that the ratio between species richness of heteroxenous vs. monoxenous parasites (i.e., parasites with complex vs. direct life cycles, respectively) can be used as a measure of pollution impact (Pérez-del Olmo et al., 2007; Sures, 2008; Sures et al., 2017b). Indeed, species richness of certain taxa (e.g., heteroxenous parasites like trematodes) has been used as a potential indicator of habitat restoration (Huspeni and Lafferty, 2004; Morley and Lewis, 2006; Sures et al., 2017a).

4. Integrating genomics, disease and conservation: current efforts and future directions

Recent advances in high-throughput sequencing technologies and concomitant development of bioinformatics tools have revolutionized the field of disease ecology. In this section, we illustrate how genomic tools have expanded the scope of wildlife disease research and refined our understanding of previously unexplored questions in disease ecology (Selbach et

al., 2019). We highlight applications of genomic tools in five key research areas and hope to bridge the gap between genomics, disease and conservation to stimulate more interdisciplinary research (figure 2.2). First, applications of emerging molecular methodologies (e.g., whole genome sequencing and metagenomics) have enhanced disease surveillance from individual hosts to ecosystem monitoring, improved management of disease outbreaks and can aid in biodiversity conservation. Second, phylogenomics can be used to infer the geographic origin of parasites, routes of parasite transmission, identify reservoir hosts, and provide more robust parasite phylogenies that can yield insights into the spatio-temporal patterns of parasite spread. Third, genomic approaches have allowed us to gain insights into fine-scale epidemiological patterns and contact tracing. Fourth, modern population genomic approaches (e.g. RAD-Seq) and genome-wide scans provide a powerful platform to examine variation in disease susceptibility, and better understand host-parasite interactions. Fifth, transcriptomics or functional genomics can help us to better uncover genetic mechanisms underlying host resistance and/or tolerance and parasite virulence, elucidating host-parasite dynamics.

4.1. Parasite detection and parasite genetic diversity

Molecular typing methods using traditional PCR techniques (e.g., genotyping or sequence analysis) have long been used to identify and characterize parasite infections as they are relatively easy to use and do not require any specialized lab setting or equipment. Quantitative PCR (qPCR) is another common diagnostic tool for sensitive and reliable identification of parasites, which also allows quantification of parasite load. More recently, the advent of droplet digital PCR (ddPCR) has advanced the detection and quantification of parasites, especially for parasites with low intensity infections (Hindson et al., 2011; Pinheiro et al., 2012). It involves absolute end-point quantification of parasites and is more advantageous

over qPCR, as it relies on calibration curves (generated from a sample with known concentration) to give relative quantification (Li et al., 2018). This technique has been successfully applied in accurate detection of several viral, bacterial diseases (King et al., 2017), and parasitic infections (Koepfli et al., 2016; Ramírez et al., 2018; Wilson et al., 2015).

Traditional PCR techniques are, however, limited in the amount of genetic information recovered as they use single or a few genetic markers. With reduced cost of high throughput sequencing, rapid development in novel genomic approaches and bioinformatics, researchers can now access high resolution genomic level information for several parasites with relative ease at a fast pace. This has revolutionized the field of molecular epidemiology, as modern genomic approaches can be used for rapid and more accurate identification of multiple parasites simultaneously and assess parasite genetic diversity at both local and global spatial scales. Whole genome sequencing and/or reduced genome technologies such as restriction-site associate DNA sequencing (RAD-SEQ) can provide thousands of markers (e.g. SNPs) for population genomics and/or phylogenomics and provide novel insights into disease transmission processes. As more parasite genomes are being sequenced, vast amounts of genomic resources have become available for downstream applications such as comparative genomics, gene expression studies and improved insights into host-parasite interactions.

Most parasite genetic studies currently focus on one or a few related parasite species. However, more powerful genomic approaches, such as metagenomics, can facilitate parasite discovery (Lipkin, 2013), and help detect newly emerging pathogens or re-emerging pathogens in novel environments. By targeting a specific genetic region (e.g., 16S, 18S rDNA), metabarcoding or amplicon-based methods have improved our capability to characterize the structure and function of parasite communities in wildlife hosts (Bergner et al., 2019; Bodewes et

al., 2014; Tanaka et al., 2014) and disease vectors (Ma et al., 2011). For example, 'nemabiome' sequencing, deep-amplicon sequencing of internal transcribed spacer 2 (ITS-2) rDNA, has been used to characterize the diversity and composition of gastro-intestinal nematode parasite communities of grazing cattle in the mid-west USA (Avramenko et al., 2015) and in wild non-human primate populations in Central Africa (Pafčo et al., 2018). In another recent study, a metabarcoding approach targeting 18S rRNA was used to characterize entire protozoan haemoparasite diversity from canine populations in Thailand (Huggins et al., 2019).

While metabarcoding has been recognized to have a great potential for parasite identification, many wildlife hosts often carry chronic infections, which makes it more difficult to obtain parasite genomic data due to the low abundance and/or intensity of parasites, without any prior enrichment strategy or when there is high host contamination. One of the common approaches aimed at increasing the probability of capturing parasite specific DNA is depletion of host DNA. For instance, Flaherty et al. (2018) undertook a novel approach of restriction enzyme digestion of host DNA prior to enrichment and 18S rRNA amplicon sequencing of blood-borne parasites in human clinical samples. This method substantially reduced human reads and increased parasitic reads 5-10 folds relative to undigested samples. Another promising approach is target capture enrichment, which utilizes parasite-specific oligonucleotide probes or baits sequences to increase the proportion of parasite-specific genomic sequences in mixed species samples (Jones and Good, 2016; Mamanova et al., 2010). For example, Lee et al. (2017) demonstrated potential use of target capture enrichment to obtain genomic sequences for multiple viral and bacterial pathogens from felids. The authors obtained up to 56 million-fold enrichment of pathogen DNA relative to the host DNA. As target capture enrichment uses probes developed from highly conserved sequences, this platform can also identify highly divergent and

previously uncharacterized pathogens, in addition to the reference pathogens used to develop capture probes (Barrow et al., 2018; Wylie et al., 2015).

Another promising application of metagenomics has been to detect and characterize all microbial taxa within hosts and better understand the relationship between host microbiome and disease. Most eukaryotic organisms host many diverse microbial communities, which can be mutualistic, commensal or parasitic symbionts (Hooper et al., 2012). Taking this simplistic classification of host symbionts as discrete ecological entities rather than viewing them as a gradient along the parasitism-mutualism continuum has been challenging (Brown et al., 2012; Ewald, 1987). Some of the key microbes that make up the resident microbiota can shift from a commensal to parasitic to mutualistic relationship in a context-dependent manner (e.g., changes in environmental conditions, tissue type, time and underlying biology of the interacting species; Leung and Poulin, 2008). An extreme example of such a transition has been observed in *Wolbachia spp*., which evolved from being a parasite (reproductive manipulator) to a mutualist (enhanced host fecundity) in natural populations of *Drosophila simulans* in over 20 years (Weeks et al., 2007). Thus, it is important to take a broad-spectrum approach when examining disease dynamics as ecological and evolutionary interactions between symbionts, both beneficial and detrimental to hosts, can influence the outcome of parasitic infections.

High throughput sequencing approaches such as metagenomics and metabarcoding have made it more feasible to characterize the host resident microbiota and its influence on host-parasitic interactions. A recent meta-analysis revealed disease associated shifts in human host microbiome, with both disease-specific and shared responses of gut microbial communities to multiple diseases (Duvallet et al., 2017). Shotgun metagenomics can also be used to investigate microbial communities in disease vectors carrying important human/wildlife vector-borne

pathogens (ticks; Carpi et al., 2011, mosquitoes; Mancini et al., 2018, triatomines; Rodríguez-Ruano et al., 2018). Such information can be leveraged to advance our understanding of host-symbiont, symbiont-symbiont interactions from the perspective of infectious disease risk and developing novel strategies for disease control (see reviews for mosquitoes; Scolari et al., 2019, sand flies; Telleria et al., 2018, triatomines; Teotônio et al., 2019). Interestingly, *Wolbachia* spp., a bacterial endosymbiont found in Drosophila fruit flies that suppresses RNA viruses has been transfected in *Aedes aegypti* mosquitoes to combat Dengue in several wild populations (reviewed in Dorigatti et al., 2018).

Host microbiome plays an important role in shaping the host immune system and can contribute to host defense against parasites (Chiu et al., 2017). A better understanding of interactions between host microbiome and parasitic infections can also aid in development of new therapeutic approaches for emerging/re-emerging diseases. The targeting of skin microbiota has gained momentum as an effective strategy against fungal pathogens. In the case of amphibian chytridiomycosis, skin microbiome is suggested to play an important role in host resistance and immune function (Bletz et al., 2013; Rebollar et al., 2016). Experimental studies have demonstrated that augmenting an antifungal bacterial species, *Janthinobacterium lividum,* to the skins of some amphibians can provide protective immunity against chytrid pathogen *Bd* and reduce morbidity and mortality of infected amphibians (Harris et al., 2009). In another study, composition of skin microbiota rather than treatment with anti-fungal bacteria improved the ability of Panamanian golden frogs to clear *Bd* infection and approximately 30% of the infected individuals survived *Bd* exposure (Becker et al., 2015). Similarly, in bats susceptible to White-nose syndrome (WNS), caused by the fungus *P. destructans*, treatment with symbiotic bacteria

(*Pseudomonas spp*.) seems to be a promising tool in protection of bats from WNS (Hoyt et al., 2015, 2019).

Taken together, these examples highlight the burgeoning potential of how host microbiome data has been invaluable for vector control strategies, tackling lethal emerging infectious diseases and improving host health and conservation. In addition to the importance of host microbiome, microbiome of parasites is important in understanding their biology and management of diseases. Despite the global concerted effort to characterize the animal microbiome through the Human Microbiome Project (Turnbaugh et al., 2007), or the Earth microbiome project (Gilbert et al., 2014), a similar initiative to characterize parasite microbiomes has only recently begun (Dheilly et al., 2017, 2019).

Moving up from within hosts to ecosystem scales, environmental DNA (eDNA) tools coupled with metagenomics, can facilitate a more in-depth investigation of diversity of parasites and characterize all parasite taxa simultaneously from bulk environmental samples (e.g., water, soil, feces). In comparison to traditional field surveys and parasitic morphological identification approaches, eDNA approaches are a powerful, non-invasive approach (see Bass et al., 2015 for review) and particularly useful for detecting parasites with complex life cycles, which tend to shed transmission stages into the environment (Sengupta et al., 2019). Although, eDNA approaches have largely been used to detect the presence/absence of free-living species and biodiversity assessments (Bohmann et al., 2014; Taberlet et al., 2012; Thomsen and Willerslev, 2015), they show promising potential in early detection of parasites, spread of invasive parasites, identifying reservoir host populations and quantifying disease risk at ecosystem scales. Until now eDNA techniques have been successfully employed to detect amphibian pathogens from aquatic ecosystems such as trematode *Ribeiroia ondatrae* (Huver et al., 2015), Ranavirus (Miaud

et al., 2019; Vilaça et al., 2019) and chytrid fungus *Bd* prior to a *Bd*-caused amphibian die-off (Kamoroff and Goldberg, 2017). Moreover, eDNA metabarcoding has also been used in early detection of disease vectors such as dipteran insects (Schneider et al., 2016) and phlebotamine sand flies (Kocher et al., 2017), which are known to transmit numerous diseases affecting human and wildlife populations.

It is, however, important to recognize that while metagenomics and/or metabarcoding approaches are promising tools for parasite characterization from host (e.g. microbiome) or environment (e.g. eDNA), its applications are still limited. In metagenomics studies, accurate parasite detection relies on the availability of comprehensive reference databases that may limit the number of parasite species and taxonomic groups detected. We still lack high quality, well-annotated reference genomes for several parasite species, especially wildlife parasites. It could also be difficult to identify some eukaryotic parasites due to its dependency on an appropriate DNA marker for metabarcoding studies. For instance, conserved genetic loci for a eukaryotic parasite may be also be conserved across its eukaryotic host and may lead to disproportionately low amplification of parasite DNA.

4.2. Parasite origin and spread

Phylogeographic analysis of pathogen genomes using metagenomics and/or whole genome sequencing offer powerful tools for identifying putative origins and evolutionary relationships among parasites (O'Hanlon et al., 2018). For instance, whole-genome sequencing of bat White-Nose fungus supports the recent introduction of *P. destructans* to North America from Europe rather than Asia and subsequent loss of genetic diversity among the isolates from North America (Drees et al., 2017). In the case of raccoon rabies virus (RRV), which has largely been restricted to eastern North America but has recently been reported from Canada (Stevenson

et al., 2016), whole-genome sequencing of RRV recovered multiple introduction events or backflow between US and Canada that resulted in multiple rabies outbreaks near the US-Canada border (Trewby et al., 2017).

Parasite genomic data can also aid in examining spatio-temporal patterns of disease spread, identifying transmission routes, and improve prediction of emerging infectious disease risk (Dellicour et al., 2016; Faria et al., 2011). For example, viral RNA-seq and whole genome sequence data have been effectively used to study the spread and transmission patterns of West Nile Virus in North America (Swetnam et al., 2018) and the spatial dynamics of the rabies virus in Africa (Brunker et al., 2018). Another novel application of pathogen genomics has been in identifying reservoir hosts. Babayan et al. (2018) analyzed viral genome sequences and used a machine learning framework to predict reservoir hosts and/or arthropod vectors for 12 different RNA viral groups based on host-associated genomic biases.

4.3. Fine scale transmission dynamics: Contact networks

At a finer spatio-temporal scale, pathogen genomic data can provide valuable, often unique insights into disease epidemiology and can be used to infer transmission trees (who-infected-whom), identify super-spreaders, elucidate host contact networks and unravel potential transmission pathways (Dudas and Bedford, 2019; Kao et al., 2014). Such information is crucial in improving our understanding of how pathogens spread within and between host populations, which in turn can be used to predict future disease risk, develop effective management interventions and potentially mitigate disease outbreaks. Whole-genome sequencing of pathogens have increasingly been used for epidemiological investigations of human health importance (Ebola virus; Gire et al., 2014, Zika virus; Thézé et al., 2018). Transmission networks in an infectious disease outbreak can now be inferred with more accuracy, via

identification of genomic variants shared between individuals (Worby et al., 2017) and SNP typing (Stucki et al., 2015).

The emerging field of phylodynamics coupled with parasite genomic data shows great potential for building upon and refining our understanding of parasite transmission in wildlife populations (see earlier section on *Spatial Structure*). Additionally, incorporating social or contact data with parasite genomic data, in a network analysis framework is an exciting open avenue for investigating disease dynamics (Gilbertson et al., 2018; White et al., 2017). For example, in a well characterized European badger and cattle population, Crispell et al. (2019) compared *Mycobacterium bovis* (causative agent for bovine tuberculosis) genomes from cattle and badgers to examine cross-species transmission and obtain fine-scale resolution of contact networks for bovine tuberculosis transmission. With pathogen genomic data, researchers were able to quantify the direction and extent of disease transmission, revealing that *M. bovis* likely transmitted more frequently from badgers to cattle (10.4x in the most likely model) and within-species transmission was more common than between-species disease transmission. It is important to note, however, that despite the greater resolution on pathogen transmission events obtained using whole genome sequencing approaches, it is still challenging to infer fine-scale epidemiological processes, especially for pathogens (e.g. bacteria) that do not evolve fast enough over epidemiological timescales, and might lack informative mutations needed to differentiate them among infected individuals (Campbell et al., 2018; Kao et al., 2014).

4.4. Host-parasite interactions: Genome wide associations

Pathogens exert a strong selective force on host populations and pathogen mediated selection on host genomes has been widely recognized across a wide range of taxa (Enard et al., 2016; Shultz and Sackton, 2019). Recent developments in high throughput sequencing have

allowed researchers to expand beyond a limited number of genes and use whole-genome scans to infer host-parasite interactions. While candidate gene approach targeting immunity related genes (MHCs, TLRs etc., see section on *Host genetic diversity*) has been the standard approach to study signatures of parasite-mediated selection on hosts, high throughput sequencing and genome-wide approaches enable an unbiased detection of areas of the genome under selection (e.g., by identifying loci that exhibit outlier allele frequencies).

Whole genome scans provide a powerful approach as it does not require *a priori* knowledge about the regions of the genome that are potentially under selection and could lead to identification of novel candidate loci associated with disease susceptibility. For instance, Epstein et al., 2016 identified two genomic regions, with putative cancer or immune function in other mammals, that may be under strong selection imposed by Devil facial tumour disease (DFTD) in Tasmanian devils, reflecting a rapid evolutionary response to infection. In another study, Cassin-Sackett et al. (2019) used a hybridization capture approach and SNP typing to identify genes under selection from avian malaria between low- and high-elevation Hawaiian amakihi population. The study revealed signatures of selection in immune-related genes and several novel candidate loci that may confer tolerance to avian malaria infection in the low-elevation amakihi populations.

4.5. Host-parasite interactions: Functional genomics

While genome wide association studies have an enormous potential to reveal novel candidate loci associated with disease susceptibility and/or resistance in natural populations, they are correlational and thus, gene expression studies are still needed to substantiate the underlying mechanisms for variation in host susceptibility to infection. Transcriptomic or RNA-seq approaches have emerged as excellent novel approaches to gain a mechanistic

understanding of host-pathogen dynamics, particularly for elucidating host response to an infection and/or identifying genes underlying parasite virulence. Host gene expression studies have major implications for elucidating genetic underpinnings of variation in host susceptibility to infection. One can also design effective disease management strategies, by identifying candidate host immune genes/pathways associated with resistance to infection and potential blocking targets for therapeutics such as virulence factors expressed by the pathogen.

RNA-seq allows sequencing of all expressed transcripts in a host organism and examine genes that may be up or down regulated in response to an infection. For instance, Videvall et al. (2015) examined host transcriptome response of European siskins (*Carduelis spinus*) infected with avian malaria parasites (*Plasmodium ashfordi*) and identified several genes (e.g., genes related to immune response, stress response) that were differentially expressed at different time-points over the course of infection. In another study examining genome-scale host gene expression patterns underlying amphibian Chytridiomycosis, researchers revealed striking differences in transcriptional response by susceptible and resistant amphibian species exposed to *Bd* (Eskew et al., 2018; Poorten and Rosenblum, 2016). The resistant amphibian host species showed minor changes in gene expression in response to *Bd* infection but exhibited an upregulation of skin structural integrity pathways compared to the susceptible species.

With further advancements in high throughput sequencing, dual RNA-seq approaches can now facilitate the simultaneous study of gene expression in hosts and pathogens and characterize genes underlying resistance/tolerance or identify virulence factors associated with the pathogen (Westermann et al., 2012). Transcriptomic studies generally generate millions of reads from the target species, but a small fraction of reads can also correspond to other non-

target species, including known or unknown pathogens, which can be easily separated by bioinformatics post-processing. For example, in the case of bat-White-nose emerging disease system, Field et al. (2015) were able to simultaneously characterize transcriptome-wide changes in gene expression in the little brown bats infected with WNS and the causative fungal agent *P. destructans*.

5. Conclusion

The importance of parasites has long been recognized, and the earliest written records of parasitic infections from ancient Egypt date back to 1500 BC (Cox 2002). However, it has only been over the last few decades that parasites have been recognized as being integral members of ecological systems, playing critical roles in many ecological, evolutionary and ecosystem-level processes. Consequently, there is a growing recognition that disease ecology can contribute substantially to the field of conservation biology. Disease ecology is a fundamentally interdisciplinary field of research and has successfully integrated theoretical models and empirical data from numerous fields, including epidemiology, parasitology, ecology, evolutionary biology and the spatial sciences (e.g., global information systems). Disease ecology has been particularly successful in utilizing the power of molecular ecology to elucidate how ecological and evolutionary factors affect, and are in turn affected by, disease dynamics in natural populations.

Modern genetic and genomic tools improve parasite diagnostics, help better understand parasite transmission and characterize aspects of the parasite (virulence genes) and host (immunogenetics) that affect disease dynamics (DeCandia et al., 2018). Consequently, the integration of disease and molecular ecology has already helped, or has the potential to help, integrate parasites into the broad framework of conservation biology in five major ways. First, at

the most basic level, molecular approaches provide an efficient way to unambiguously identify parasites and quantify infection intensities (see above); Second, genetic approaches provide critical information to elucidate co-evolutionary dynamics between symbiotic species, such as hosts and parasites. Such information is especially important from a conservation perspective because, in the ongoing mass extinction, parasites and mutualists may comprise some of the most vulnerable organismal groups (Derne et al., 2018; Dunn et al., 2009). Third, molecular tools in conjunction with population genetic and genomic analyses have provided critical insights into how anthropogenic changes, such as global climate change and habitat modification, are likely to affect parasite transmission dynamics (see details above). Additionally, modern technologies (e.g., next-generation sequencing) and novel analytical approaches (e.g., phylodynamics and network analyses) are allowing conservation biologists to leverage parasite genetics (e.g., bacterial transmission networks) to obtain vital information on host ecology (e.g., social structure and movement patterns) that can be used to inform conservation action (see details above); Fourth, both traditional (e.g., Sanger sequencing) and modern (e.g., next-generation sequencing) provide an excellent platform to further empirical studies of parasites at ecosystem levels, and thus have the potential to improve our understanding of the role parasites play in ecosystem structure and function, and aid the further the development of parasites as indicators of ecosystem health (see details above); Finally, conservation biology has long recognized the importance of biodiversity (Soulé, 1985) due to its intrinsic value (Ghilarov, 2000) and its importance from a utilitarian anthropocentric perspective (e.g., maintenance of ecosystem function and provision of ecosystem services; Justus et al., 2009; Mace et al., 2012). A large proportion of Earth's biodiversity is likely represented by parasitic species (Poulin and Morand, 2000). It is estimated that helminth parasites alone have 50% more species than there are

vertebrate hosts (Dobson et al., 2008), but only about ten percent of parasite species have been documented (Brooks and Hoberg, 2013), and it remains impossible to quantify the exact number of parasitic species. However, molecular tools can be effectively used to uncover cryptic parasite diversity and can thus be used to set conservation priorities through the development of unbiased metrics, such as those based on phylogenetic diversity (Faith, 2008; Mooers et al., 2008; Nunes et al., 2015; Winter et al., 2013). Consequently, there remains a great scope for genetic and genomic tools to help incorporate parasites into the broader discussion on species diversity, and potentially help elevate parasites to conservation end-points in and of themselves.

6. Acknowledgements

We thank colleagues at SREL for help and support. Financial support was provided from the U.S. Department of Energy Financial Assistance Award no. DE-FC09-96SR18546. VVR was supported by intramural funds from Indian Institute of Science Education and Research (IISER) Tirupati. Figures in the manuscript were created in Mind the Graph and Biorender platforms.

7. Figures

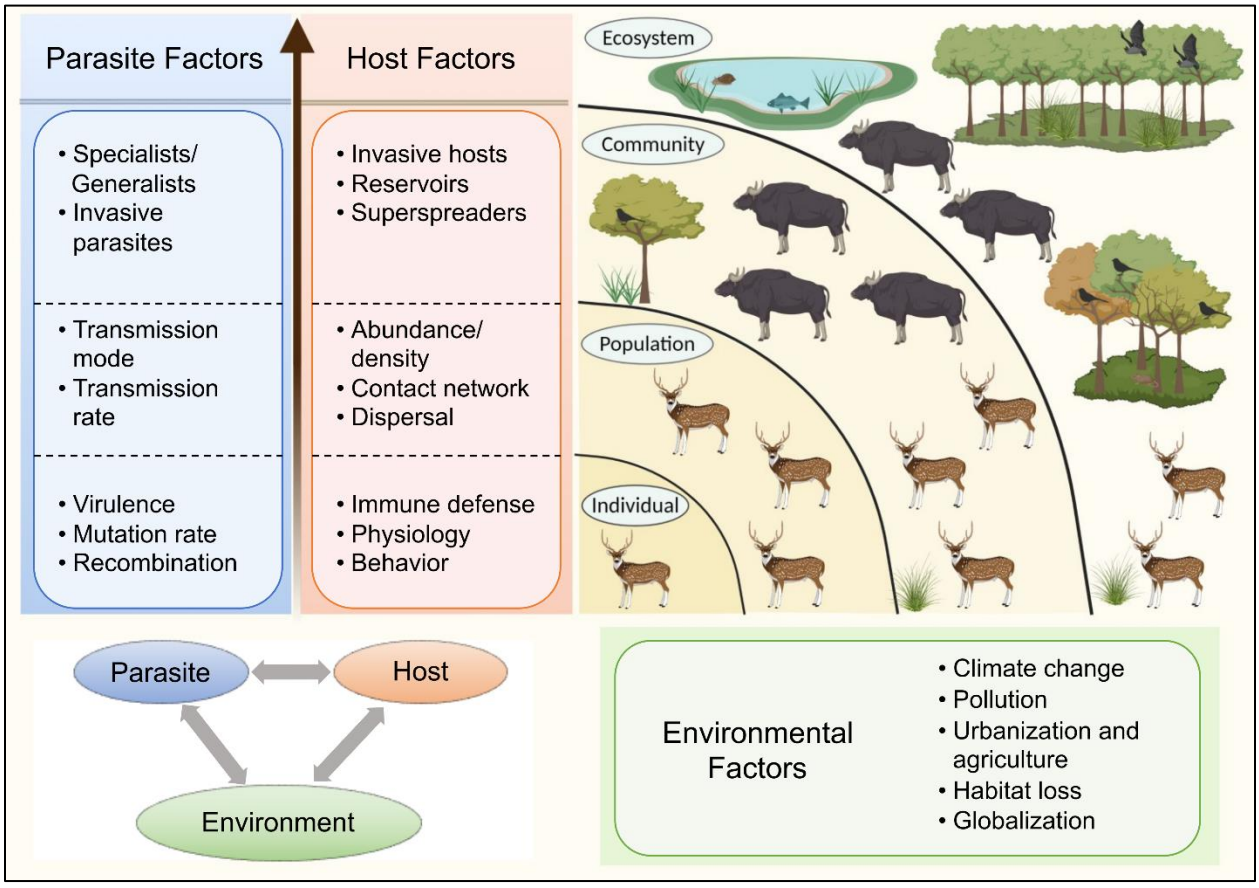


Figure 2.1. Schematic illustrating host, parasite and environmental factors influencing disease dynamics across scales of biological organization, at the individual, population, community, and ecosystem scales. It is noteworthy that the effect of host and parasite factors on disease dynamics at each level is often not mutually exclusive and can range from individual- to population- and community/ecosystem levels.

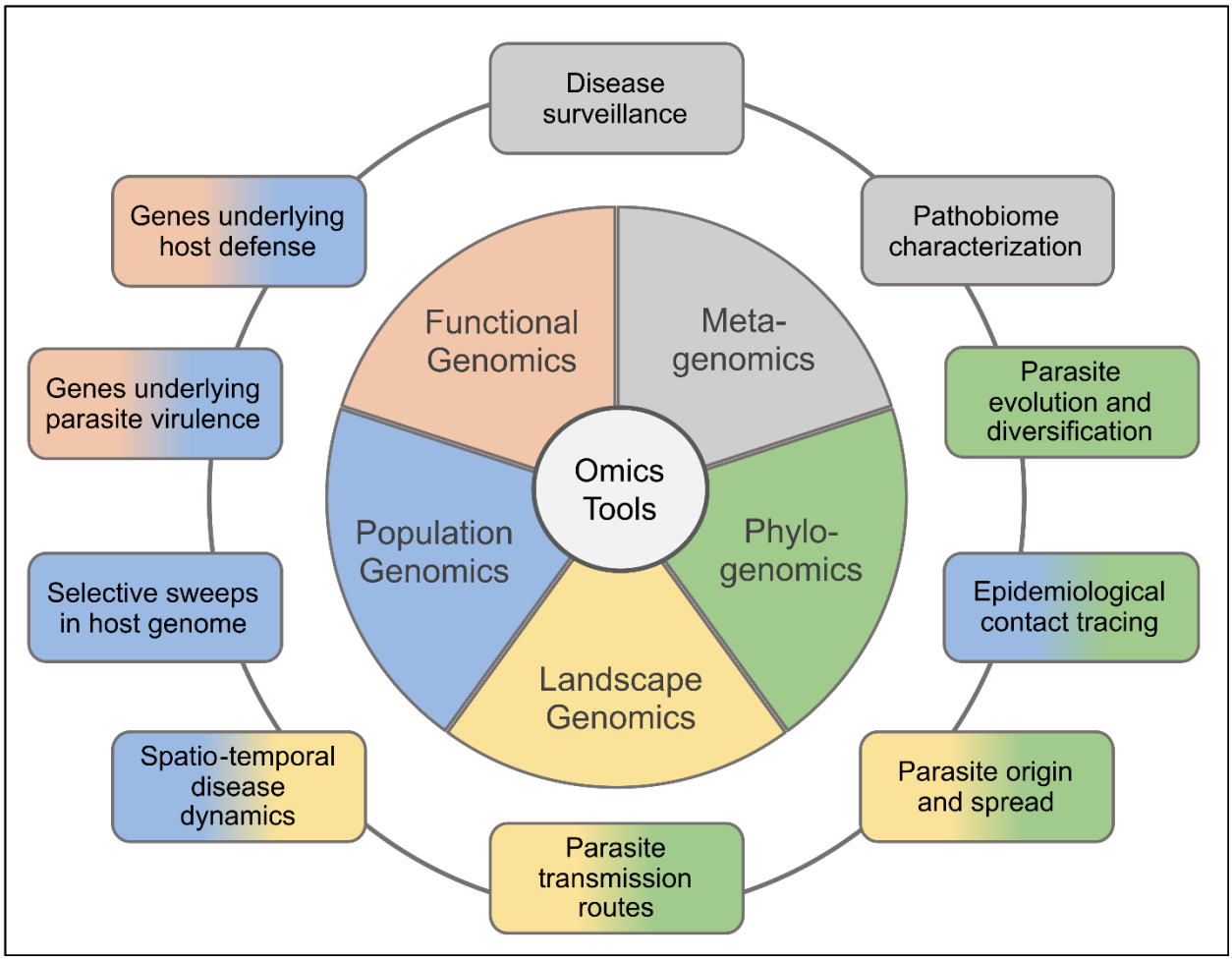


Figure 2.2. Applications of various high-throughput genomic tools (inner circle) to answer key questions in disease ecology (outer circle). Each genomic tool is represented by a different color and color gradients in the outer circle represent the multiple genomic approaches that can be used to answer a given question in the box. For instance, parasite origin and spread can be inferred using Landscape genomics and/or Phylogenomics.

CHAPTER 3

GEOGRAPHIC AND HOST SPECIES BARRIERS DIFFERENTIALLY AFFECT GENERALIST AND SPECIALIST PARASITE COMMUNITY STRUCTURE IN A TOPICAL SKY-ISLAND ARCHIPELAGO [2]

[2] **Gupta, P.**, Vishnudas, C. K., Ramakrishnan, U., Robin, V. V., & Dharmarajan, G. (2019). Geographical and host species barriers differentially affect generalist and specialist parasite community structure in a tropical sky-island archipelago. *Proceedings of the Royal Society B, 286*(1904). DOI: https://doi.org/10.1098/rspb.2019.0439. Reprinted here with permission of publisher.

Abstract

Understanding why some parasites emerge in novel host communities while others do not has broad implications for human and wildlife health. In the case of haemosporidian blood parasites, epidemic wild bird mortalities on oceanic islands have been linked to *Plasmodium* spp., but not genera like *Haemoproteus*. Indeed, Haemoproteus is absent from many oceanic islands. By contrast, birds on continental islands share long coevolutionary histories with both *Plasmodium* and *Haemoproteus*, and are thus ideal model systems to elucidate eco-evolutionary endpoints associated with these parasites in oceanic islands. Here, we examine eco-evolutionary dynamics of avian haemosporidian in the Shola Sky Island archipelago of the Western Ghats, India. Our analyses reveal that compared to *Plasmodium*, *Haemoproteus* lineages were highly host-specific and diversified via co-speciation with their hosts. We show that community structure of host-generalist *Plasmodium* was primarily driven by geographical factors (e.g. biogeographic barriers), while that of host-specialist *Haemoproteus* was driven by host species barriers (e.g. phylogenetic distance). Consequently, a few host species can harbour a high diversity of *Plasmodium* lineages which, in turn, are capable of infecting multiple host species. These two mechanisms can act in concert to increase the risk of introduction, establishment, and emergence of novel *Plasmodium* lineages in island systems.

Keywords: avian haemosporidians, *Plasmodium*, *Haemoproteus*, disease emergence, community structure, India

1. Introduction

Emerging infectious diseases are considered to be one of the greatest challenges of our times from the perspective of human and wildlife health, as well as ecosystem function and stability (Preston et al., 2016; Rogalski et al., 2017). An important driver for the dramatic increase in disease emergence over the past several decades is the recent and rapid spread of parasites outside their native range due to myriad factors including global climate change and increased human-mediated transport (Rogalski et al., 2017). Such parasite range expansion can lead to serious epidemics in naïve host populations into which these parasites are newly introduced (Dunn and Hatcher, 2015; Lips et al., 2006; Tompkins et al., 2003).

Avian haemosporidians (Apicomplexa: Haemosporida; *Plasmodium* and other related genera such as *Haemoproteus* – hereafter avian malaria) are a globally distributed group of vector-borne blood parasites that infect a wide array of bird taxa (Valkiŭnas, 2005). Avian malaria caused by *Plasmodium* spp. is one of the most important emerging infectious diseases of wild bird populations globally (Marzal et al., 2015; Niebuhr et al., 2016; Schoener et al., 2014). Large-scale mortalities in native wild birds have been well documented due to accidental introduction of *Plasmodium* spp. and *Culex quinquefaciatus* into island bird communities which had no coevolutionary history with these parasites (e.g., Hawaii; Valkiŭnas, 2005; Warner, 1968 and New Zealand; Niebuhr et al., 2016; Schoener et al., 2014). However, similar epidemic mortalities by *Haemoproteus* spp. have not been recognized. Indeed, while *Plasmodium* spp. are cosmopolitan (Clark et al., 2014), *Haemoproteus* spp. only appear to have colonized some oceanic islands systems (e.g. Lesser Antilles; Fallon et al., 2005; Svensson-Coelho and Ricklefs, 2011) and is absent from many others (e.g., Hawaii, New Zealand and French Polynesia; Clark et al., 2014; Fecchio et al., 2019b; Niebuhr et al., 2016).

The reduced ability to colonize some islands by *Haemoproteus* spp. vs. *Plasmodium* spp., and consequently the lower negative consequences associated with parasite invasions on native bird communities, are likely driven by myriad factors such as parasite specialization and avian host/vector community composition. Previous studies indicate that *Plasmodium* spp. are relatively generalist, infecting a wide range of host species, whereas *Haemoproteus* spp. generally exhibit specialist associations and are restricted to phylogenetically related host species (Beadell et al., 2009; Clark et al., 2014; Fecchio et al., 2017; Ishtiaq et al., 2007), but this pattern is not universal (Fecchio et al., 2019b; Moens and Pérez-Tris, 2016; Svensson-coelho et al., 2013). Such eco-evolutionary differences likely affect the ability of generalist parasites, like *Plasmodium* spp., to readily establish in island communities when introduced by natural or anthropogenic factors (Clark et al., 2014; Ewen et al., 2012). However, the taxonomic distinctiveness of host communities on islands may protect them from invasions by specialist parasites, like *Haemoproteus* spp., if island communities consist of species phylogenetically distant to hosts in the parasite's native range. Consequently, the colonization history of avian hosts/vectors, that is specific to each island system, can critically affect the likelihood of colonization by specialist parasites, such as *Haemoproteus* spp., but not generalist ones, such as *Plasmodium* spp.

Understanding the underlying eco-evolutionary mechanisms that influence the colonization and maintenance of *Plasmodium* spp. vs. *Haemoproteus* spp. can help elucidate the drivers of disease emergence in natural communities. In this context, continental Sky Islands are a fascinating model system because they provide excellent natural laboratories for examining parasite eco-evolutionary dynamics. Sky Islands are isolated montane forests surrounded by a "sea" of low-elevation habitat, limiting dispersal of both bird and parasite lineages, similar to

oceanic islands (Mccormack et al., 2009). Thus, Sky Island bird communities may face many of the same eco-evolutionary challenges as their oceanic counterparts. However, Sky Island bird communities, in contrast to many oceanic counterparts, have generally shared long coevolutionary histories with their parasites. Consequently, the bird communities on continental Sky Islands can help elucidate the potential long-term ecological and evolutionary end-points for oceanic island bird communities where avian haemosporidians have recently been introduced.

Here, we examine the eco-evolutionary dynamics of avian haemosporidians in the Sky Island archipelago of the Western Ghats, Southern India. These Sky Islands (hereafter Shola Sky Islands) are high elevation montane ecosystems characterized by unique habitats called *Sholas*, a natural mosaic of wet, tropical evergreen forests and grasslands, isolated by drier lowland habitats (Robin and Nandini, 2012). The Shola Sky Islands harbor remarkable species diversity and endemism driven by geographic complexity at multiple spatial scales (Myers et al., 2000; Robin and Nandini, 2012). At large spatial scales (i.e., across the Western Ghats), the deep and wide biogeographic barriers (Chaliyar, Palghat and Shencottah gaps; figure 3.1) have led to avian lineage diversification (Robin et al., 2015b, 2017). At small spatial scales (i.e., individual mountains) the steep elevational gradient contributes to colonization of Sky Islands by both specialist avian species restricted to montane habitats and generalists with a wide elevational range. Thus, the Shola Sky Islands offer an excellent opportunity to better understand the relative importance of geographic (e.g., spatial distance and biogeographic gaps), climatic (e.g., elevational gradients) and host species barriers (e.g., host phylogeny and host ecology) in driving evolution of parasite community structure.

In this study, we test whether *Plasmodium* spp. and *Haemoproteus* spp., due to their varying levels of host specialization differ in terms of: (i) host association patterns, (ii)

coevolutionary dynamics, (iii) genetic structure, and (iv) global phylogenetic structure. We predict that: (i) diversity of hosts infected by a single lineage would be greater for generalist vs. specialist parasites; (ii) generalist parasites would likely coevolve with hosts through host-switching, while specialists would likely co-speciate with their hosts; (iii) genetic structure of generalist parasites would primarily be influenced by geography, while specialists would be more affected by host species barriers; and (iv) phylogenetic structure at global scales would be lower for the generalist vs. specialist parasites because geographical range tends to correlate positively with niche breadth (Slatyer et al., 2013).

2. Materials and methods

2.1. Field and laboratory methods

Field sampling was conducted at 7-14 sites across four major geographical regions in the southern 600 km mountain range of the Western Ghats (at 100-2500 m.a.s.l.; figure 3.1; see also Appendix A, S3.1). Each geographical region corresponded to the Sky Island group separated by three biogeographical barriers—Chaliyar River valley, Palghat Gap and Shencottah Gap. Adult birds were captured using mist-nets during 2011-2013 and blood samples were collected from bird's ulnar vein in Queen's lysis buffer, following Robin et al. (Robin et al., 2010). Genomic DNA was extracted using Qiagen blood and tissue extraction kit (Qiagen, Hilden, Germany) and screened for haemosporidian infection by amplifying 478 bp of mitochondrial cytochrome *b* gene (cytb) of avian haemosporidian parasites (Hellgren et al., 2004; details in Appendix A, Methods).

2.2. Phylogenetic analyses

To assess phylogenetic relationships among the Shola Sky Island haemosporidian parasite lineages, we conducted Bayesian phylogenetic analyses in MrBayes (Ronquist and

Huelsenbeck, 2003). Similarly, we built host phylogenetic tree based on cytochrome *b* sequence data (1143bp) for bird species from an earlier study (Robin et al., 2015b). To examine parasite phylogenetic relationships at the global scale, we obtained cytochrome *b* sequence data from the MalAvi database (Bensch et al., 2009; accessed February 2018) and built bayesian parasite phylogenetic trees in MrBayes (Ronquist and Huelsenbeck, 2003). We calculated rarefaction curves of expected phylogenetic diversity for host species and parasite lineages to ensure adequate sampling, as implemented in R-package 'pdcalc' (Nipperess and Matsen, 2013; details in Appendix A, Methods). All statistical analyses were carried out in R 3.6.2 (R Core Team 2019), unless specifically mentioned otherwise.

2.3. Host-parasite association patterns

We measured the diversity of parasite lineages infecting each host species and diversity of hosts infected by each parasite lineage using the Shannon diversity of interactions index (H2; Rzanny and Voigt, 2012), a two-dimensional equivalent of the Shannon index (Blüthgen et al., 2008). We built null models by randomizing the network interactions (10,000 times) while maintaining the marginal sums (i.e. sum of interactions for each species was kept constant) using R-package vegan (Oksanen et al., 2016). We performed two-sided tests of the network metric value against the distribution of the null model metric values to assess statistical significance. We quantified host specialization for parasite lineages infecting ≥ 2 host species by measuring the phylospecificity index—mean phylogenetic distance (MPD) and standardized effect sizes of the MPD values (SES.MPD; Poulin et al., 2011; Webb, 2000) using R-package picante (Kembel et al., 2010; details in Appendix A, Methods).

2.4. Host-parasite coevolutionary dynamics

We visually assessed phylogenetic congruence between the host and parasite phylogenetic trees by constructing a cophylogenetic tanglegram using TREEMAP (Charleston et al., 2002). We then statistically tested for host-parasite phylogenetic congruence by conducting a distance based co-phylogenetic analyses in Procrustean Approach to Cophylogeny (PACO) (Balbuena et al., 2013), as implemented in R-packages 'ape' and 'vegan' (Oksanen et al., 2016; Paradis and Schliep, 2019). We also conducted an event-based co-phylogenetic analyses, as implemented in Jane (Conow et al., 2010) and Core-PA (Merkle et al., 2010), to determine the type and frequency of different coevolutionary scenarios, e.g., co-speciation, duplication, host switch, sorting or loss of parasite lineages. While jane assigns an *a priori* cost for each evolutionary event, core-pa does not require *a priori* assignment of cost values to compute a cost minimal reconstruction. Run parameters and settings are detailed in the Appendix A, Methods.

2.5. Parasite genetic structure

To test whether parasite genetic structure was influenced by host species barriers, biogeographic gaps (see figure 3.1) and geographical structure within each biogeographical region, we used a hierarchical Analysis of Molecular Variance (AMOVA) as implemented in the R-package 'hierfstat' (Goudet, 2005). We assessed the statistical significance of each variance estimate by conducting 1000 randomizations amongst species (for $F_{Host/Total}$), regions within each species (for $F_{Region/Host}$), and sampling sites within regions (for $F_{Site/Region}$).

Furthermore, we tested the relative effects of geographic, climatic and host factors on parasite genetic structure using Multiple Regression on Distance Matrices (MRM) (Lichstein, 2007), as implemented in **'**ecodist' (Goslee and Urban, 2015). The geographic factors considered were biogeographic gaps (as a Boolean matrix) and geographical distance (i.e., the great circle

distance between sampling coordinates); climatic factors included elevational distance (i.e., absolute difference in elevation between sampling sites); host factors included host phylogenetic and host ecological distance (measured as Gower distance between host ecological traits; (Gower, 1971). Host ecological data included species traits that could affect haemosporidian infection dynamics and were collected from published sources (Wilman et al., 2014), as well as field observations by VVR and CKV (see Appendix A, S3.9).

2.6. Global parasite phylogenetic structure

To test whether specialist vs generalist parasites were phylogenetically more clustered across the global haemosporidian phylogeny, we calculated the nearest neighbor phylogenetic distance (D_{KN}) within *Plasmodium* spp. and *Haemoproteus* spp. lineages. We produced a null distribution of D_{KN} values by randomizing (1000 times) tip labels across the global phylogeny and calculated the probability of obtaining a simulated D_{KN} value $\leq$ observed D_{KN} value. We tested the overall significance (i.e. across lineages within each parasite genus) using the exact binomial test in R.

3. Results and Discussion

3.1. Parasite prevalence patterns

We sampled 1177 birds belonging to 28 species (including 14 endemics), representing almost the entire Shola Sky Island bird community (except two species, see Appendix A, S3.2) and found 24 species (490 birds) infected with haemosporidians (41.6% prevalence; figure 3.1). *Plasmodium* spp. was found at a prevalence of 13.6% (across 19 bird species), while *Haemoproteus* spp. had a prevalence of 68.9% (across 20 bird species; Appendix A, S3.2). Haemosporidian prevalence varied across species, with *Turdus merula* as a key host species for *Plasmodium* spp. infection (29% prevalence) and *Zosterops palpebrosus* for *Haemoproteus* spp.

(77.1% prevalence) infection. Rarefaction analyses revealed that our sampling was adequate to recover the observed parasite phylogenetic diversity (Appendix A, S3.14). Among the 47 parasite lineages recovered, a majority of *Plasmodium* spp.(10 of 18) and *Haemoproteus* spp. (24 of 29) lineages were novel and unique to the Shola Sky Islands (Appendix A, S3.3), indicating that many haemosporidian lineages are generally restricted to a single biogeographic region and characterized by local diversification as suggested by Ellis et al. (Clark, 2018; Ellis et al., 2018; Fecchio et al., 2019a).

3.2. Host-parasite association patterns

Plasmodium spp. and *Haemoproteus* spp. differed markedly in terms of host-parasite associations, with two *Plasmodium* spp. lineages infected a greater diversity of hosts than expected by chance (P_MSP02: Observed H2 = 1.748; Expected H2 = 0.806; $P = 0.016$; P_MSP03: Observed H2 = 2.246; Expected H2 = 0.795; $P < 0.001$; figure 3.2; Appendix A, S3.4). However, patterns of generalist host-parasite associations were not statistically significant across all *Plasmodium* spp. lineages (Binomial $P = 0.058$). Additionally, while host individuals were not susceptible to a greater diversity of *Plasmodium* spp. lineages than expected by chance (Binomial $P = 0.340$), it is important to note that a disproportionately high diversity of *Plasmodium* spp. lineages (7 of 18) were recovered from a single host species – *Turdus merula* (Observed H2 = 1.715; Expected H2 = 0.978; $P = 0.046$; figure 3.2; Appendix A, S3.6). In contrast, for *Haemoproteus* spp., there was a strong positive association between hosts and parasite lineages, with 27 of 29 parasite lineages infecting a lower diversity of hosts (Binomial $P < 0.001$; Appendix A, S3.5) and 23 of 24 host species being infected by a lower diversity of parasites than expected by chance (Binomial $P < 0.001$; figure 3.2, Appendix A, S3.6).

Furthermore, phylogenetic host specificity analyses for parasite lineages infecting multiple host species revealed higher host specialization for *Haemoproteus* spp. (MPD_w mean = 0.132, CI = 0.038, 0.248) compared to *Plasmodium* spp. lineages (MPD_w mean = 0.358, CI = 0.246, 0.443). While four of seven *Haemoproteus* spp. lineages showed higher phylospecificity (based on their significant SES.MPD values), none of the *Plasmodium* spp. lineages had higher host specificity than expected by chance (Appendix A, S3.7). Thus, *Haemoproteus* spp. were highly host specialized, with most lineages infecting one or a very few phylogenetically clustered hosts, compared to *Plasmodium* spp., as observed in other biogeographic regions (Beadell et al., 2009; Clark et al., 2014; Fecchio et al., 2017; Ishtiaq et al., 2007; Olsson-Pons et al., 2015).

Interestingly, high prevalence and diversity of *Plasmodium* spp. lineages were recovered from a single host species – *Turdus merula*. Based on existing genetic data and plumage-based taxonomy, *T. merula* is known to harbor cryptic species diversity, with overlapping ranges of resident and migratory races (Rasmussen and Anderton, 2005; Robin et al., 2015b), which may explain why it was infected by diverse haemosporidian lineages. Additionally, *T. merula* harbored some widespread and pathogenic haemosporidian lineages, which may underscore its role as potential reservoir host in the Shola Sky Island bird community. Among the eight *Plasmodium* spp. lineages infecting *T. merula*, one was a generalist while others were restricted to *T. merula* and two lineages matched FANTAIL01 and GRW06 (*Plasmodium elongatum*) (Appendix A, S3.3). While FANTAIL01 is relatively less common, GRW06 is globally widespread and often virulent in naïve bird hosts (Palinauskas et al., 2016). Moreover, out of the three *Haemoproteus* spp. lineages detected in *T. merula*, one matched and two were 99% similar to *Haemoproteus minutus*, a widespread European lineage of *Turdus* spp. While *Haemoproteus*

minutus is relatively benign for native European birds, lethal outbreaks have been recorded for naïve captive parrots in Europe (Ortiz-Catedral et al., 2019; Palinauskas et al., 2008). Previous studies have also shown that Eurasian blackbird and other thrushes (*Turdus* spp.) generally serve as key reservoir hosts for *Plasmodium* spp. infections with high prevalence and diversity in continental communities; and contribute to high spillover risk to naive host communities when introduced to islands (such as in Azores; Hellgren et al., 2011), Robinson Crusoe (Martínez et al., 2015) and New Zealand (Niebuhr et al., 2016)). Thus, *T. merula* could be a potential key reservoir host in the Western Ghats with several virulent lineages.

3.3. Host-parasite coevolutionary dynamics

We found no evidence of significant cophylogenetic congruence between hosts and *Plasmodium* spp. phylogenies (PACo, $m^2 = 5.297$, $P = 0.640$) but there was significant cophylogenetic congruence between host and *Haemoproteus* spp. phylogenies (PACo, $m^2 = 7.39$, $P = 0.047$; see also Appendix A S3.15, S3.16). Co-phylogenetic analysis with jane revealed significant topological congruence between host and *Plasmodium* spp. or *Haemoproteus* spp. phylogenies (optimal inferred reconstruction cost lower than expected by chance; $P < 0.001$; Appendix A, S3.17, S3.18). However, core-pa revealed co-speciation for *Haemoproteus* spp., with inferred co-speciation events significantly greater than expected by chance ($P = 0.05$) while other host-switching, sorting or duplication events did not differ significantly from random expectations. For *Plasmodium* spp., none of the events occurred significantly more than expected by chance (Appendix A, S3.8).

Overall, as expected, our cophylogenetic analyses revealed a signal of host-parasite congruence mediated by co-speciation for specialist *Haemoproteus* spp., but lack of congruence for the generalist *Plasmodium* spp. The significant role of co-speciation vs. host-switching in the

evolutionary history of *Haemoproteus* spp. in the Shola Sky Islands is in contrast to previous studies that recognize host switching as the dominant coevolutionary mechanism (Fecchio et al., 2018; Ricklefs et al., 2014, 2004). Our study suggests that coevolutionary mechanisms underlying diversification of avian haemosporidians are likely more complex than has been anticipated earlier. Employing a probabilistic approach such as approximate Bayesian computation (ABC) represent useful future directions for an improved understanding of avian haemosporidian diversification as has been proposed recently (Alcala et al., 2017).

The specialist strategy of *Haemoproteus* spp. and history of co-speciation may have facilitated its diversification in the Shola Sky Island bird community. For example, three specialist lineages – MONCAC03, MONFAI02, MONMER02 showed signal of co-speciation and have co-diversified with their endemic hosts *Montecincla cachinnans*, *Montecincla fairbanki* and *Montecincla meridionalis*, respectively (see Appendix A, S3.18). Our results further strengthen the patterns of local diversification of avian haemosporidians observed in other tropical bird communities (Ellis et al., 2018; Fecchio et al., 2019a). Broadly, empirical data from other host-parasite systems suggests that parasites tend to be host-specialists in species rich communities (Krasnov et al., 2008). Similarly, in the highly diverse Shola Sky Island bird communities with old host evolutionary histories and many endemic host radiations, parasites likely benefit by establishing host-specialized associations and diversify by co-speciation rather than adapting a generalist strategy and having more opportunities for host-switching, as suggested earlier (Fecchio et al., 2018; Moens and Pérez-Tris, 2016).

3.4. Parasite genetic structure

Analysis of Molecular Variance (AMOVA) revealed that parasite genetic differentiation between host species was low for *Plasmodium* spp. ($F_{Host/Total} = 0.073$, $P = 0.045$) and high for

Haemoproteus spp. ($F_{Host/Total}$ = 0.688, *P* = 0.001; figure 3.3; Appendix A, S3.10). We found a significant effect of biogeographic gaps, within host species on the genetic structure of *Plasmodium* spp. ($F_{Region/Host}$ = 0.208, *P* = 0.004) but not *Haemoproteus* spp. ($F_{Region/Host}$ = 0.031, *P* = 0.464). However, there was significant parasite genetic structure between sampling sites within biogeographical regions for both *Plasmodium* spp. ($F_{Site/Region}$ = 0.079, *P* = 0.007) and *Haemoproteus* spp. ($F_{Site/Region}$ = 0.113, *P* = 0.018; figure 3.3; Appendix A, S3.10).

Furthermore, Multiple Regressions on Distance Matrices (MRM) analyses showed that *Plasmodium* spp. parasite genetic distance was significantly associated with biogeographic gaps (B = 0.229, t = 4.686, P = 0.003) and geographic distance (B = 0.094, t = 4.425, *P* = 0.002) but not with host phylogenetic (B = 0.020, t = 0.809, *P* = 0.592), ecological (B = 0.023, t = 0.656, *P* = 0.609) or elevational distance (B = -0.027, t = -1.101, *P* = 0.360; figure 3.3; Appendix A, S3.11). Alternatively, *Haemoproteus* spp. parasite genetic distance was significantly associated with host phylogenetic (B = 0.059, t = 18.157, *P* = 0.014), ecological (B = 0.164, t = 44.794, *P* = 0.001) and elevational distance (B = 0.053, t = 16.614, *P* = 0.037), but was not affected by biogeographic gaps (B = 0.017, t = 3.114, *P* = 0.558) or geographic distance (B = 0.001, t = 0.389, *P* = 0.909; figure 3.3; Appendix A, S3.11).

From an eco-evolutionary perspective, parasites are intrinsically tied to their hosts and may be affected by host phylogeography. Thus, given the effect of biogeographic gaps in the Western Ghats on host phylogeographic structure, we expected to find similar phylogeographic structure among the parasite lineages. Indeed, at large spatial scales, *Plasmodium* spp. lineages revealed phylogeographic structure across the biogeographic gaps. Surprisingly, *Haemoproteus* spp. structure was not affected by biogeographic gaps, suggesting that these parasites tend to track their hosts closely and have likely colonized their hosts before hosts genetic divergence. It

was especially surprising that even host species (e.g., *Sholicola* spp. and *Montecincla* spp.) that showed deep genetic divergence (~4-5 Ma; Robin et al., 2017) across the biogeographic gaps were infected by similar *Haemoproteus* spp. lineages across their range. This could likely occur due to differences in mutation rates of parasites compared to their hosts. Additionally, an open and interesting question remains regarding the role of the dipteran vectors in facilitating dispersal of *Haemoproteus* spp. lineages across the biogeographic gaps.

Within a biogeographical region, we found that *Plasmodium* spp. lineages were shared more among geographically closer hosts and did not show any host phylogenetic or ecological constraints, coherent with their generalist strategy and a characteristic that likely contributes to its role as an emerging parasite in novel bird communities. In contrast, specialist *Haemoproteus* spp. lineages were shared more among closely related hosts (phylogenetically and ecologically), despite their geographical isolation, a finding consistent with earlier studies (Fecchio et al., 2017; Olsson-Pons et al., 2015). Interestingly, *Haemoproteus* spp. populations were structured by elevation compared to *Plasmodium* spp., indicating a higher probability of elevational spread by *Plasmodium* spp., which has critical implications from the perspective of disease emergence in novel climatic niches.

Broadly, our results provide interesting insights into how hosts may be analogous to islands from the perspective of parasite's colonization (Kuris et al., 1980). For instance, in the case of *Plasmodium* spp., biogeographic gaps influenced parasite genetic structure, indicating that host communities in each Sky Island group served as islands. In contrast, host phylogenetic and ecological differences constrained the dispersal of *Haemoproteus* parasites, thus characterizing each host species as islands.

3.5. Global parasite phylogenetic structure

We found that phylogenetic clustering in *Plasmodium* spp. lineages from the Shola Sky Islands did not differ from a random sample of lineages from the global parasite pool at the community or lineage level (Mean D_{KN} = 0.533, P = 0.272; 2 of 18 lineages had D_{KN} lower than expected; figure 3.4; Appendix A, S3.12). In contrast, *Haemoproteus* spp. lineages showed strong phylogenetic clustering at both community and lineage level (Mean D_{KN} = 0.281, P = 0.002; 14 of 29 lineages had D_{KN} less than expected; figure 3.4; Appendix A, S3.13). Overall, *Haemoproteus* spp. lineages had a significantly higher chance of being clustered compared to *Plasmodium* spp. lineages ($\beta \pm SE = 1.958 \pm 0.86$, Odds ratio = 7.086; $z = 2.277$, P = 0.023).

In line with our expectations, the generalist *Plasmodium* spp. lineages were widely interspersed across their global phylogeny whereas specialist *Haemoproteus* spp. lineages were phylogenetically more clustered. This suggests that *Haemoproteus* spp. have likely diversified in the Western Ghats, owing to the relatively old origin (Praveen Karanth, 2015) and the deep evolutionary history of Western Ghats endemic avian hosts (Robin et al., 2015b) such as *Sholicola* spp. and *Montecincla* spp., which diverged from their most recent ancestor about 11-12 Ma and later diversified on the Shola Sky Islands about 4-5 Ma (Robin et al., 2017). Lack of phylogenetic clustering among the *Plasmodium* spp. lineages suggests that these parasites are a random sample of their global phylogenetic pool and remain unconstrained by host phylogeny, further highlighting their potential as emerging parasites in novel host communities.

4. Conclusion

We present one of the first comprehensive investigation of avian haemosporidian dynamics in the Indian subcontinent by sampling almost the entire bird community in an important biodiversity hotspot(see also (Ishtiaq et al., 2017). Here, we addressed the differential

effects of geographic, climatic and host species barriers in shaping generalist and specialist haemosporidian parasite community structure. Our results reveal that, in a continental island system with long host-parasite coevolutionary history, there were several novel haemosporidian parasite lineages, endemic to the Shola Sky Islands. *Plasmodium* spp. and *Haemoproteus* spp. clearly differed in terms of their host diversity, with higher host specialization in the case of the latter but not in the former. Consequently, there was a strong signal of co-speciation in the coevolutionary history of *Haemoproteus* spp., but not in *Plasmodium* spp. These parasites also differed dramatically in terms of their emerging infectious disease risk, with sharing of generalist *Plasmodium* spp. lineages among multiple host species primarily constrained by geographic factors such as geographic proximity, whereas specialist *Haemoproteus* spp. lineages were more influenced by host species factors such as host phylogeny, host ecology and climatic factors driven by elevation. Critically, our analyses revealed that *Plasmodium* spp. were less affected by climatic gradients (i.e., elevation), indicating that these parasites had a higher likelihood of elevational range expansion and were more likely to emerge when introduced to novel environments. In the Shola Sky Islands, this is an especially troubling finding as high elevation habitats harbor higher number of endemic host species, which are also more likely to have evolved with avian haemosporidian parasites (for example, see Niebuhr et al., 2016).

Overall, our results reveal that the higher likelihood of emergence in novel host communities by *Plasmodium* spp. vs. *Haemoproteus* spp. was likely driven by two interrelated mechanisms. First, there are a few *Plasmodium* spp. lineages that can infect a diverse array of host species without being constrained by host phylogenetic/ecological similarity, and thus these lineages could emerge rapidly when introduced into a novel host community. Second, a few host species harbor a high diversity of *Plasmodium* spp. lineages, and thus invasion of such hosts into

a novel bird community will be associated with the introduction of multiple parasite lineages, increasing the likelihood of spill-over to native hosts. Consequently, *Plasmodium* spp. lineages were globally widespread, reiterating their increased potential for colonization and emergence in novel host communities. Elucidating the underlying ecological and evolutionary factors that contribute to the rapid emergence of some parasites (e.g. *Plasmodium* spp.) but not others (e.g. *Haemoproteus* spp.) has critical implications for an improved understanding of emerging infectious diseases.

5. Acknowledgements

We thank: Permits—Forest Departments of Kerala (R. Rajaraja Varma, V. Gopinathan, T. M. Manoharan and B. S. Corrie) and Tamilnadu (R. Sunderaraju); GIS data— M. Arasumani; laboratory work—Anuradha Chandramouli; comments on manuscript—Farah Ishtiaq.

6. Funding

Financial support was provided from the U.S. Department of Energy Financial Assistance Award no. DE-EM0004391 to the University of Georgia Research Foundation. This work also was funded by Ramanujan Fellowship from Department of Science and Technology, India to GD, National Geographic Society Research and Exploration grant to V.V.R., DAE award to study Indian Biogeography, Ramanujan Fellowship and NCBS internal funding to U.R.

7. Figures

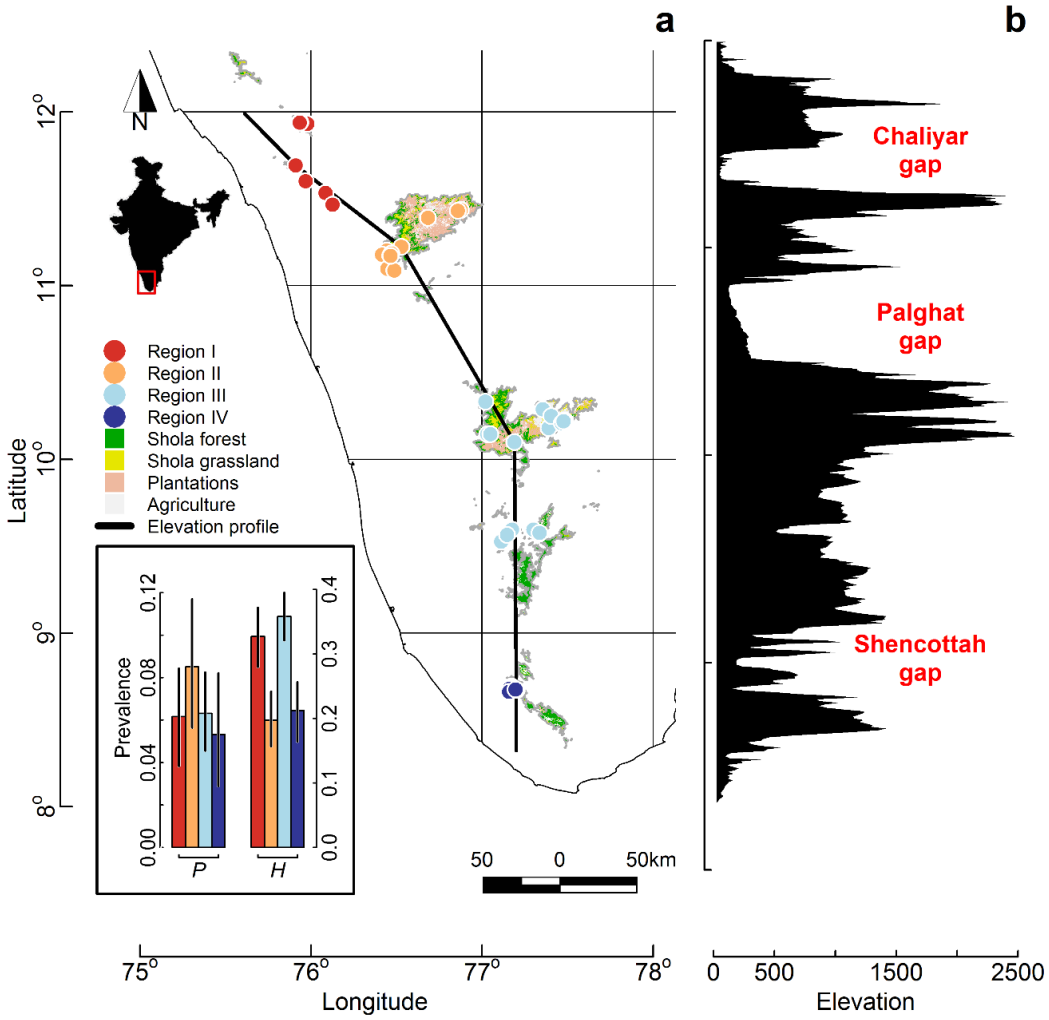


Figure 3.1. Map of Western Ghats. (a) Locations of sampling sites (filled circles) in four geographical regions: I (Bababudan & Banasura hills), II (Nilgiri hills), III (Anamalai-Palni-Highwavies Hills), IV (Ashambu hills), corresponding to the major Sky Island group separated by three biogeographical barriers—Chaliyar Gap, Palghat Gap and Shencottah Gap. Underlying natural (i.e. forest and grassland) vs. plantation habitats and 1400 msl isoclines are also depicted. Inset shows the proportion of individuals infected with *Plasmodium* spp. and *Haemoproteus* spp. in each geographical region with their 95% bootstrap confidence intervals; **(b)** Elevation profile of the Western Ghats along a linear transect connecting the highest elevation points in each geographical region (black transect line in A).

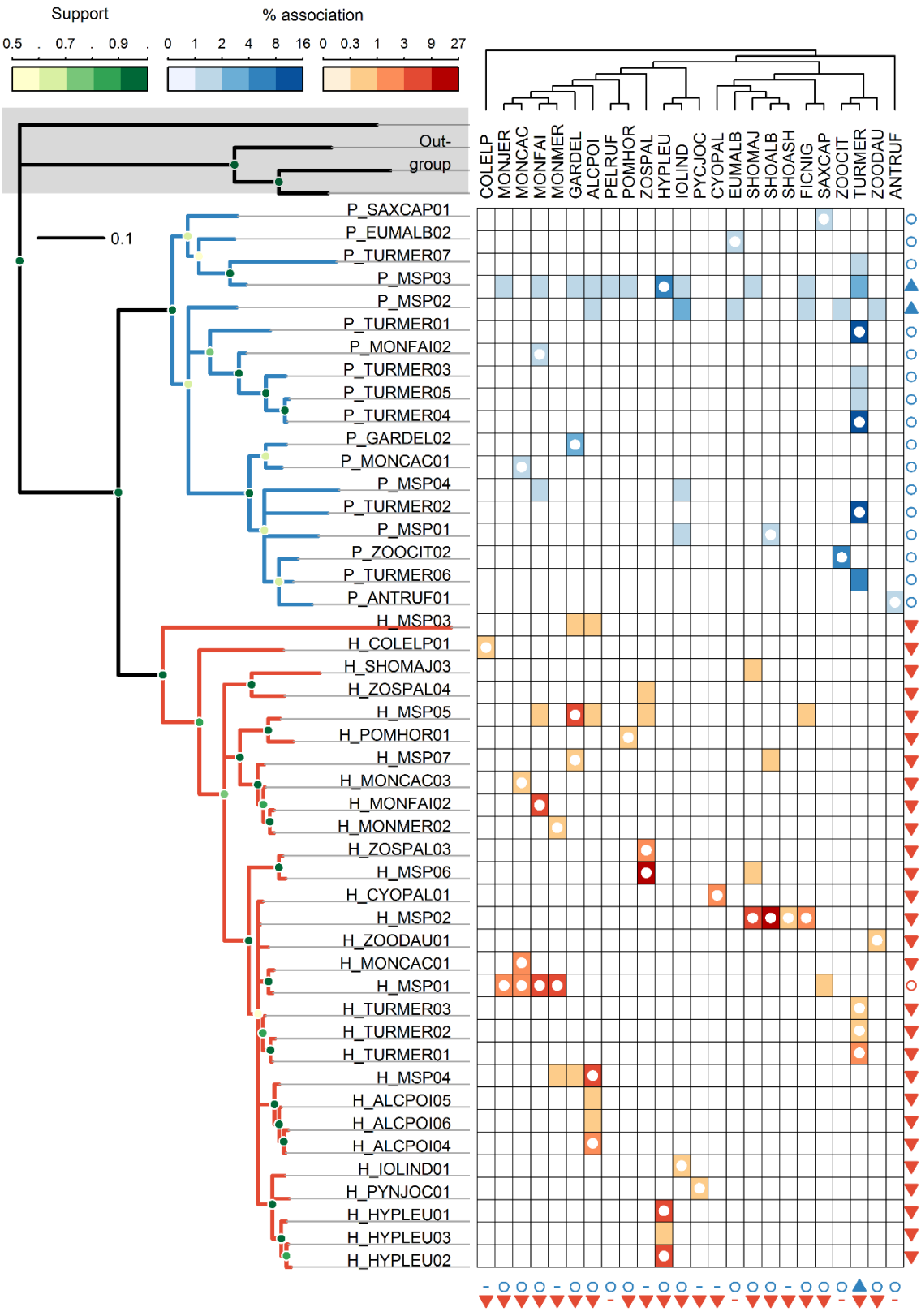

Support
0.5 . 0.7 . 0.9 .
% association
0 1 2 4 8 16
0 0.3 1 3 9 27
Out-
group
0.1
COLELP
MONJER
MONCAC
MONFAI
MONMER
GARDEL
ALCPOI
PELRUF
POMHOR
ZOSPAL
HYPLEU
IOLIND
PYCJOC
CYOPAL
EUMALB
SHOMAJ
SHOALB
SHOASH
FICNIG
SAXCAP
ZOOCIT
TURMER
ZOODAU
ANTRUF
P_SAXCAP01
P_EUMALB02
P_TURMER07
P_MSP03
P_MSP02
P_TURMER01
P_MONFAI02
P_TURMER03
P_TURMER05
P_TURMER04
P_GARDEL02
P_MONCAC01
P_MSP04
P_TURMER02
P_MSP01
P_ZOOCIT02
P_TURMER06
P_ANTRUF01
H_MSP03
H_COLELP01
H_SHOMAJ03
H_ZOSPAL04
H_MSP05
H_POMHOR01
H_MSP07
H_MONCAC03
H_MONFAI02
H_MONMER02
H_ZOSPAL03
H_MSP06
H_CYOPAL01
H_MSP02
H_ZOODAU01
H_MONCAC01
H_MSP01
H_TURMER03
H_TURMER02
H_TURMER01
H_MSP04
H_ALCPOI05
H_ALCPOI06
H_ALCPOI04
H_IOLIND01
H_PYNJOC01
H_HYPLEU01
H_HYPLEU03
H_HYPLEU02

Figure 3.2. Host-association matrix for avian haemosporidians in the Shola Sky Islands. Left: Bayesian phylogenetic tree of *Plasmodium* spp. (blue) and *Haemoproteus* spp. (red) lineages based on cytochrome b gene sequence data, with *Leucocytozoon* spp. as outgroups. Bayesian posterior probability support values are color coded. Top: Bayesian phylogenetic tree of Shola Sky Island bird species. See Appendix A S3.2, S3.3 for details on tree tip labels. The network matrix represents the heat map of abundance and distribution of each *Plasmodium* spp. and *Haemoproteus* spp. lineage, ranging from cool blues/reds (low abundance) to warm blues/reds (high abundance), respectively. White circles in the colored cells indicate significance of the of the network metric value against null expectations. Triangles depicted on the edges of the matrix indicate significant values of Shannon diversity of interactions (two-tailed test), circles show non-significance and dashes indicate an absence of infection.

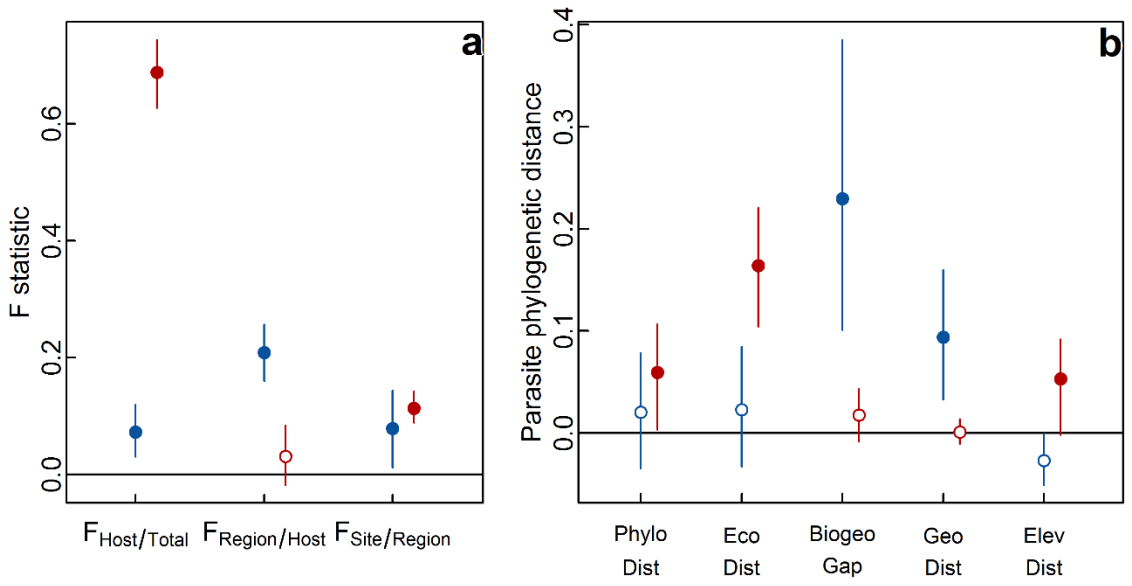


Figure 3.3. Biogeographic structuring of Shola Sky Island haemosporidian lineages. **(a)** Analysis of Molecular Variance (AMOVA) representing the effects of host species barriers ($F_{Host/Total}$), biogeographic regions within host species ($F_{Region/Host}$), and geographic site within each biogeographic region ($F_{Site/Region}$) on parasite genetic structure for *Plasmodium* spp. (blue) and *Haemoproteus* spp. (red). **(b)** Multiple Regression on distance Matrices (MRM) analysis representing the effects of host phylogenetic distance (Phylo Dist), host ecological distance (Eco Dist), biogeographic gaps (Biogeo Gap), geographic distance (Geo Dist) and elevational distance (Elev Dist) on parasite phylogenetic structure for *Plasmodium* spp. (blue) and *Haemoproteus* spp. (red). Filled symbols indicate *F* values significantly different from random expectation with their 95% bootstrap confidence intervals.

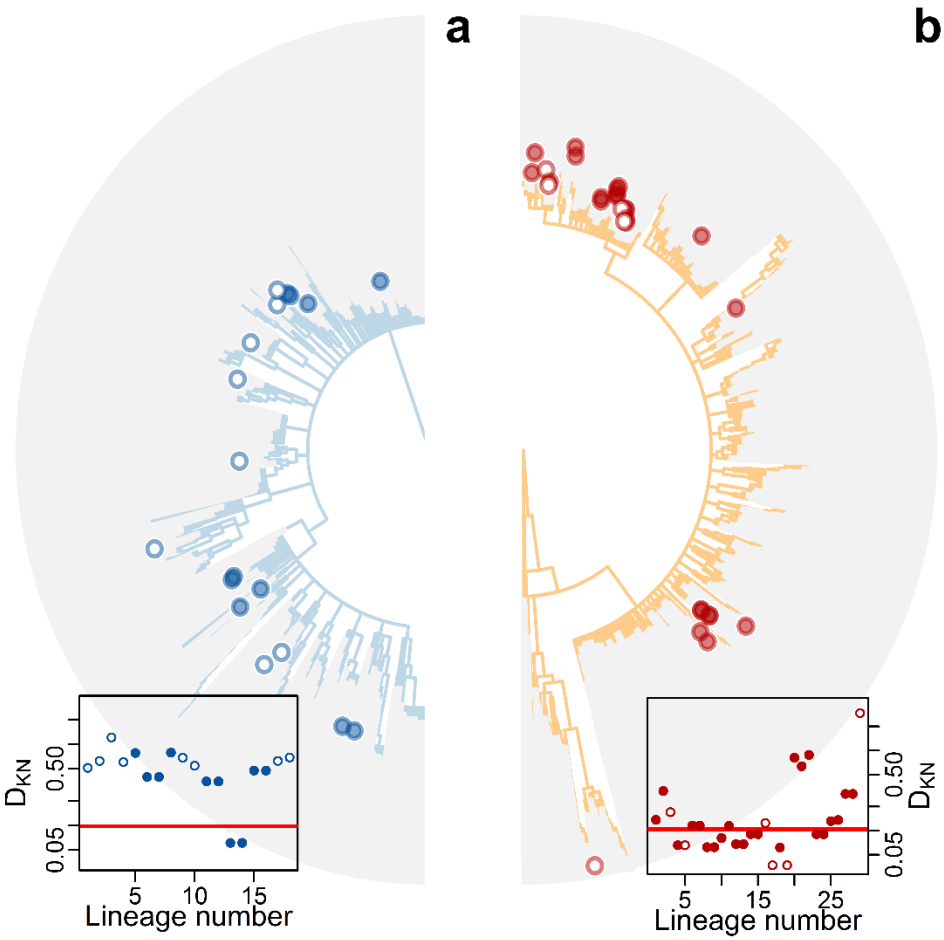


Figure 3.4. Global phylogenetic structure based on nearest neighbor phylogenetic distance (D_{KN}). Bayesian phylogenetic trees for **(a)** *Plasmodium* spp. and **(b)** *Haemoproteus* spp. lineages based on cytochrome *b* gene sequence data obtained from MalAvi database and endemic (closed circles) and non-endemic (open circles) lineages recovered from the Shola Sky Islands. *Leucocytozoon* spp. were used as an outgroup. Inset shows the observed (circles) and expected (line) nearest neighbor phylogenetic distance (D_{KN}) for each Shola Sky Island haemosporidian lineage.

CHAPTER 4

HOST PHYLOGENY MATTERS: EXAMINING SOURCES OF VARIATION IN INFECTION RISK BY BLOOD PARASITES ACROSS A TROPICAL MONTANE BIRD COMMUNITY IN INDIA[3]

[3] **Gupta, P.**, Vishnudas, C. K., Robin, V. V., & Dharmarajan, G. (2020). Host Phylogeny Matters: Examining Sources of Variation in Infection Risk by Blood Parasites Across a Tropical Montane Bird Community in India. Submitted to *Parasites and Vectors*. DOI: https://doi.org/10.21203/rs.3.rs-33026/v1

Abstract

Background: Identifying patterns and drivers of infection risk among host communities is crucial to elucidate disease dynamics and predict infectious disease risk in wildlife populations. Blood parasites of genera *Plasmodium* and *Haemoproteus* are a diverse group of vector-borne protozoan parasites that affected bird populations globally. Despite their widespread distribution and exceptional diversity, factors underlying haemosporidian infection risk in wild bird communities remain poorly understood. While some studies have examined variation in avian haemosporidian risk, researchers have primarily focused on host ecological traits without considering host phylogenetic relationships. In this study, we employ a phylogenetically informed approach to examine the association between host ecological traits and avian haemosporidian infection risk in endemic bird communities in the Western Ghats Sky Islands.

Methods: We collected blood samples from 1177 birds (28 species) and amplified partial parasite mitochondrial cytochrome b gene to identify avian haemosporidian infection and characterized unique haemosporidian lineages by sequencing. We employed a Bayesian phylogenetic mixed effect modelling approach to test the association between seven species specific ecological predictors, four individual level predictors and avian haemosporidian infection risk. We also examined the effect of host phylogenetic relationships on the observed patterns of variation in haemosporidian infection risk by estimating phylogenetic signal.

Results: Our study shows that effects of host ecological traits and host phylogeny on infection risk vary for *Plasmodium* (generalist parasite) vs. *Haemoproteus* (specialist parasite). For *Plasmodium*, we found that sociality, sexual dimorphism and feeding strata were important ecological predictors. For *Haemoproteus*, patterns of infection risk among host species were associated with sociality, elevation and individual body condition. Interestingly, variance in

infection risk explained by host phylogeny was higher for *Haemoproteus* parasites compared to *Plasmodium*.

Conclusion: Our study highlights that while host ecological traits promoting parasite exposure and host susceptibility are important determinants of infection risk, host phylogeny also contributes substantially in predicting patterns of avian haemosporidian infection risk among host communities. Importantly, infection risk is driven by joint contributions of host ecology and host phylogeny and studying these effects together could increase our ability to better understand the drivers of infection risk and predict future disease threats.

Keywords: Avian haemosporidians, Plasmodium, Haemoproteus, ecological traits, host phylogeny, infection dynamics, Western Ghats, India

1. Introduction

Identifying factors that determine the variation in disease risk in natural populations is of fundamental importance for understanding the ecology and evolution of host-parasite interactions and predicting infectious disease risk. In multi-host, multi-parasite systems, host species can vary substantially in infection risk and heterogeneity in disease risk among hosts can be driven by individual- or species-level host characteristics. At the species-level, variation in infection risk can occur because of differences in host-life history, behavior and environment that underpin patterns of parasite exposure (Ezenwa et al., 2016; Herrera and Nunn, 2019; Johnson et al., 2012; Parham et al., 2015). At the individual-level, hosts can vary in infection risk owing to differences in exposure to parasites and host susceptibility. In the case of vector-borne diseases, hosts exposure to parasites can increase via increase in frequency of encounter with dipteran vectors that can influence disease transmission (Medeiros et al., 2015). For instance, host exposure can be impacted by geographical factors that affect vector abundance (e.g. elevation; Lapointe et al., 2012) or hosts ecological traits that affect exposure risk such as foraging/nest height (Garvin and Remsen, 1997; González et al., 2014) or sociality (Arriero and Moller, 2008; Fecchio et al., 2011). Infection risk may also be impacted by factors associated with disease susceptibility such as species-specific ecological traits (e.g. sexual dimorphism; Moller, 1990) or individual-level factors associated with fitness (e.g. fluctuating asymmetry; Beasley et al., 2013; Møller, 1992) and body condition (Merino et al., 2012; Sánchez et al., 2018).

Avian haemosporidian parasites (Apicomplexa, Haemosporida) of the genera *Plasmodium*, *Haemoproteus* (including *Parahaemoproteus*) are protozoan blood parasites that affect bird populations globally (Valkiŭnas, 2005). Avian haemosporidians (commonly referred to as avian malaria parasites) are an exceptionally diverse group of parasites, with over 2500

parasite genetic lineages (Bensch et al., 2009). Avian haemosporidians are transmitted by arthropod vectors, with *Plasmodium* being transmitted by Culicid mosquitoes, and *Haemoproteus* by Ceratopogonid biting midges and Hippoboscid louse flies (Santiago-Alarcon et al., 2012; Valkiŭnas, 2005). Avian haemosporidians can impose strong selective pressures on bird hosts as they can reduce longevity (Asghar et al., 2015), host fitness (Asghar et al., 2011; Lachish et al., 2011), individual condition (Schrader et al., 2003), and lead to population declines (Argilla et al., 2013; Atkinson and Samuel, 2010; Dadam et al., 2019; Levin et al., 2009). Previous research has revealed that avian haemosporidian parasites vary widely in their host range, with *Plasmodium* lineages often being generalists infecting a broad range of host species and *Haemoproteus* lineages often being specialists infecting one or few closely related host species (Clark et al., 2014; Gupta et al., 2019). *Plasmodium* and *Haemoproteus* parasites also exhibit eco-evolutionary differences, with *Plasmodium* more affected by abiotic factors such as geography and *Haemoproteus*, primarily affected by biotic factors such as host phylogeny and host ecology (Gupta et al. 2019). Given their widespread distribution, diversity and pronounced eco-evolutionary differences between *Plasmodium* and *Haemoproteus*, different ecological factors may explain variation in parasite prevalence for the two parasite genera.

The tropical Sky Island bird community in the Western Ghats mountains – located parallel to the southern coast of India, offer an excellent model system to elucidate the factors influencing variation in avian haemosporidian infection risk. The Western Ghats are a global biodiversity hotspot (Myers et al., 2000), and the high endemic bird diversity in the Western Ghats (Robin and Nandini, 2012) provides opportunities for native parasites to exploit a wide variety of hosts, allowing us to test how host ecology impacts disease risk. Additionally, the landscape is threatened by anthropogenic habitat fragmentation and land-use changes; and the

potential negative impact of avian malaria in this biodiversity hotspot makes the identification of factors associated with increased disease risk an important step for conservation (Grogan et al., 2014).

Sky Islands are isolated mountain-top habitats surrounded by dramatically different lowland habitats. The replicated arrangement of geographically discrete, identical habitats provides an ideal natural laboratory to explore ecological dynamics underlying avian haemosporidian infection risk. The Western Ghats Sky Islands hosts a unique natural matrix of wet, montane evergreen forests— locally known as *Sholas*— and grasslands above 1400 m ((hereby Shola Sky Islands), while low elevations harbor drier habitats. High habitat heterogeneity and climactic conditions due to its elevational gradient have led to disproportionately high host species diversity in the Sky Islands, comprising of host species having different habitat specialization, life history strategies and elevational distribution. For example, montane specialists are restricted to high elevations and generalists are distributed widely from high to low elevations. While montane specialists have historically been protected from avian malaria because low temperatures at high elevations leads to low vector abundance (Lapointe et al., 2012) or poor parasite development (LaPointe et al., 2010), this scenario is changing as global warming progresses (Altizer et al., 2013). Thus, Western Ghats Sky Islands offer a valuable system in which to investigate disease dynamics, especially in the light of climate change driven extinctions in the landscape (e.g., Robin et al., 2014).

Although several factors have been proposed to explain variation in parasite prevalence and infection risk among individuals and host species (Garvin and Remsen, 1997; González et al., 2014; Illera et al., 2017; Lutz et al., 2015; Ricklefs et al., 2005; Scheuerlein and Ricklefs, 2004), it remains unclear whether the role of host ecological traits are generally predictable or

whether they are idiosyncratic across hosts, parasites, and environmental conditions and context dependent. Additionally, few studies have taken evolutionary history of the hosts into account and thus, the importance of host evolutionary history in predicting infection risk is poorly understood. Evolutionary history of the host species can confound the relationship between ecological traits and parasite infection risk as closely related species are more likely to share risk factors compared to non-related host species (Barrow et al., 2019).

In this study, we employ a phylogenetically controlled approach to examine which host ecological and morphological factors influence variation in avian haemosporidian prevalence and thus avian haemosporidian infection risk, by sampling almost the entire sky island bird communities in the Western Ghats. We also assess whether these effects differ across the two parasite genera – *Plasmodium* and *Haemoproteus*. We expect that in the Western Ghats system where *Plasmodium* is a generalist parasite and *Haemoproteus* is a relatively specialist parasite, the effects of ecological factors will vary for *Plasmodium* and *Haemoproteus*, in addition to their intrinsic differences in parasite biology and vector specificity. We expect that at the species-level, 1) Species that have a lower minimum elevation will have higher *Plasmodium* prevalence whereas species with a higher minimum elevation will have higher *Haemoproteus* prevalence because of different environmental requirements of haemosporidian parasites that may limit their distribution on an elevational gradient; 2) Species foraging at higher forest strata will have lower *Plasmodium* prevalence and higher *Haemoproteus* prevalence compared to species foraging at the ground level, because of vertical stratification in their arthropod vectors; 3) Social living species will likely exhibit higher parasite prevalence of both parasites as social living species may have a higher probability of encountering vectors and increase transmission risk; 4) Host species with sexual dimorphism will exhibit higher haemosporidian prevalence as a result of

parasite- mediated sexual selection. Furthermore, at the individual-level, 5) birds with higher average body size will have a higher probability of infection for both parasites as larger body size will likely provide more surface area for vector feeding and emit higher quantity of olfactory cues (e.g. CO_2), thereby attracting more vectors; 6) birds with better body condition will likely be less infected by both parasites compared to birds with poorer body condition.

2. Methods

Study area and collection of parasite data

We sampled at 52 localities across four major sky island groups spanning 600 km in the southern Western Ghats mountain range during 2011-2013 (figure 4.1). We captured birds using mist-nets and collected blood samples (50-100 μl) from the ulnar vein of the bird with a heparinized micro-hematocrit capillary tube and immediately stored in blood lysis buffer. We used genetic data for *Plasmodium* and *Haemoproteus* parasites generated in an earlier study (details in Gupta et al., 2019). Briefly, avian haemosporidian infection was identified by amplifying parasite's partial mitochondrial cytochrome b gene (478bp) (Hellgren et al., 2004). Positive infections were sequenced and paired DNA sequences were aligned in GENEIOUS 9.1.5 (Kearse et al., 2012). Unique haemosporidian lineages were identified by comparing parasite sequences with publicly available sequences in NCBI and in the MalAvi database (Bensch et al., 2009).

Ecological and morphological trait data

We collected data on host ecological traits based on the current understanding of vectors transmitting avian haemosporidians and included traits that increase hosts' exposure to parasites. Data on ecological traits of species was collected from previous field observations by CKV and VVR and Wilman et al. (2014). Our dataset included seven species-specific categorical

variables: foraging strata (High/Low), roosting behavior (Social/Non-social), host habitat type (Forest/Grassland), elevational range (Specialist/Generalist), genetic connectivity (Breaks/No breaks), sexual dimorphism (Yes/No) and minimum species elevation as a covariate (Appendix B, S4.1, S4.2). At the individual level, the morphological trait data consisted of four variables associated with body size, fluctuating asymmetry (FA) and body condition. The body size variables included tarsus and wing measurements (Appendix B, S4.2). We calculated a measure of fluctuating asymmetry with respect to tarsus (FA_{Tarsus}) as per Van Dongen (1999). We also estimated individual body condition, a commonly used proxy of infection-induced fitness cost (Sánchez et al., 2018), based on scaled mass index $\widehat{M}$, as proposed by Peig and Green (2009), which accounts for covariance between body size and body mass components. The condition score was calculated by standardizing body mass at a fixed value of a linear body measurement based on the scaling relationship between mass and length. We used body weight as the mass measurement and wing length measurement as the length variable. All individual measurement variables were standardized by a z-transform within each species (i.e. a unit increase in the measurement indicates one standard deviation increase over the mean value for the species).

Statistical Analyses

We built Bayesian phylogenetic mixed models (BPMM) to assess the association between infection risk and host ecological and morphometric traits using the R- package 'MCMCglmm' (Hadfield, 2010). We used BPMM as it allowed us to control for non-independence of trait data due to host phylogenetic relationships (Garamszegi, 2014). We modeled host infection status as a binary response variable (0 for uninfected, 1 for infected) with a logit link, for *Plasmodium* and *Haemoproteus*, and different species ecological traits and individual morphological traits as predictor variables. To account for shared ancestry between

hosts, we fit a variance–covariance matrix of phylogenetic distances between host species generated from the host phylogeny as a random effect. We used host phylogeny based on cytochrome *b* sequence data (1143bp) from earlier studies (Gupta et al., 2019; Robin et al., 2015b). We included sampling sites as another random effect to account for non-independence among the sampled individuals due to sampling design. We conducted two separate BPMM analyses with the host species ecological traits and individual trait data because we had complete morphometric measurements for only a subset of individuals (n = 991 individuals). We excluded all individuals without complete information from the individual level BPMM analysis. For both datasets, we first tested a fully parameterized model including all predictors and then ran subsequent reduced models by excluding non-significant predictors, one at a time based on p-values. We used weak, uninformative prior (normal distribution with mean of zero and very large variance) for the fixed effects, an expanded prior (χ 2 distribution with 1 degree of freedom) for the random effects and fixed residual variance at 1, based on recommendations by de Villemereuil et al. (2013) and Hadfield (2010). We ran each model chain for 2 million iterations with burn-in of 100,000 and thinning intervals of 1000 iterations. Additionally, we conducted three independent MCMC runs for our final reduced model that included significant predictors from both species- and individual-level analyses. Analyses for each parasite genus (*Plasmodium* and *Haemoproteus*) were conducted separately.

We visually analyzed the trace plots for all model parameters to assess mixing properties and stationarity of chains. We assessed convergence of the MCMC chains by evaluating correlation between samples (autocorrelation <0.1) and Gelman-Rubin statistic (Potential scale reduction factor, PSRF < 1.1 preferred among chains) using R-package 'coda' (Plummer et al., 2006). We considered model parameters to be significant when the 95% credible intervals (CIs)

of posterior estimates excluded zero and p-values were < 0.05. Furthermore, we estimated phylogenetic heritability, equivalent to Pagel's lambda (λ), as a measure of degree of phylogenetic signal and calculated as the proportion of the total variance explained by the species phylogeny (Hadfield and Nakagawa, 2010; Pagel, 1999). We estimated the mean and 95% highest posterior density (HPD) of λ for each MCMC chain by dividing the phylogenetic variance-covariance (VCV) matrix by the sum of the phylogenetic, location, and residual VCV matrices (Hadfield & Nakagawa 2010). All statistical analyses and graphing were conducted in R ver. 3.6.2 (R Core Team 2019).

3. Results

Avian haemosporidian prevalence

Our dataset included 1177 birds across 28 bird species, representing almost the entire Shola sky island bird community (Appendix B, S4.1). We found 24/28 bird species infected (490 birds, 41.6% prevalence) with avian haemosporidian parasites. Among the 47 unique haemosporidian lineages, 10/18 *Plasmodium* and 24/29 *Haemoproteus* lineages were novel and endemic to the Shola Sky Islands (Gupta et al., 2019). Haemosporidian prevalence varied across host species, with *Turdus merula* exhibiting high *Plasmodium* prevalence (29%, N=86) and *Zosterops palpebrosus* showing high *Haemoproteus* prevalence (77.1%, N=118).

Species ecology and individual body condition affects avian haemosporidian prevalence

Some ecological predictors we tested were unimportant for infection status responses (i.e., the 95% CI overlapped with 0) and were removed to construct the reduced models (Appendix B, S4.3). As expected, different ecological predictors were important for variation in infection risk by *Plasmodium* and *Haemoproteus* (figure 4.2). At the species level, sociality and sexual dimorphism were positively associated with *Plasmodium* prevalence (β=2.56, CI=0.35,

4.89) and (β=3.17, CI=0.96, 5.34), respectively. Additionally, species foraging at high strata had lower *Plasmodium* prevalence (β=-3.25, CI=-4.87, -1.31) compared to low strata foragers. For *Haemoproteus*, sociality and species elevation were significant predictors of *Haemoproteus* parasite prevalence in the Western Ghats sky island bird community (figure 4.2, Appendix B, S4.3). Social roosting species had higher *Haemoproteus* prevalence (β=4.99, CI=2.64, 7.67) compared to non-social species. Minimum elevation of host species had a significant positive association with *Haemoproteus* prevalence (β=0.64, CI=0.32, 0.97).

Among the individual level predictors, we did not find significant relationship between the various morphometric measurements (tarsus and wing lengths), fluctuating asymmetry, body condition and variation in haemosporidian prevalence for *Plasmodium* parasites. But our final model for *Haemoproteus* revealed individual body condition as a significant predictor for *Haemoproteus* prevalence (figure 4.2, Appendix B, S4.3). We found that *Haemoproteus* prevalence increased significantly with birds having better body condition (β=0.59, CI=0.04, 1.10). All other predictors revealed no significant relationship with *Haemoproteus* parasite prevalence.

Furthermore, we recovered phylogenetic signal in both our full and reduced models, in the case of both *Plasmodium* and *Haemoproteus*; however, phylogenetic signal was lower for *Plasmodium* compared to *Haemoproteus*. After taking into account the variation explained by host ecological traits, location effects and residual variance, host species phylogeny explained 48% (β=8.33, CI=2.99, 14.40) of the total variation observed in *Haemoproteus* prevalence and 27% (β=4.80, CI=0.88, 8.98) of the total variation in *Plasmodium* prevalence across host species (figure 4.3, Appendix B, S4.4).

4. Discussion

In this study, we show that multiple host ecological factors are important determinants of avian haemosporidian infection risk across avian hosts in the Western Ghats sky island bird community. However, these effects varied among *Plasmodium* and *Haemoproteus* parasites, likely due to their eco-evolutionary differences and vector preferences. Previous studies have also reported mixed support for the ability of host ecological factors to predict avian haemosporidian prevalence(González et al., 2014; Illera et al., 2017; Lutz et al., 2015; Scheuerlein and Ricklefs, 2004). This suggests these patterns are far from universal and underlying host community structure and/or host evolutionary history plays a key role in assessing avian haemosporidian infection risk.

We hypothesized that prevalence of *Haemoproteus* parasites will likely increase with species' elevation as cooler temperatures at higher elevations may support the survival and development of both *Haemoproteus* parasites and their associated vectors (i.e. biting midges; Berenger and Parola, 2017). We lend support to this hypothesis as our results show that species elevation was significantly associated with *Haemoproteus* prevalence, with species at higher minimum elevation having higher *Haemoproteus* prevalence compared to species at lower minimum elevation. This agrees with previous studies that had found support for higher *Haemoproteus* prevalence at higher elevations with low temperatures (Harrigan et al., 2014; Illera et al., 2017; Pérez-Rodríguez et al., 2013). In the light of global climate change, our findings indicate that *Haemoproteus* parasites, which are currently more prevalent at higher elevations, might undergo range collapse due to unavailability of suitable environment (niche) for its survival and development (Pérez-Rodríguez et al., 2014). In addition to the environmental constraints on *Haemoproteus* parasites, the observed patterns could also be confounded by

specific host species present at high elevation as *Haemoproteus* parasites tend to be host specialists in the Western Ghats (Gupta et al., 2019).

Our findings indicate that host species ecological traits that promote exposure risk likely explain the increased prevalence of avian haemosporidian parasites. Several studies have found evidence for higher avian haemosporidian prevalence in social birds (Tella, 2002; but see Arriero and Moller, 2008). Among the various host ecological traits tested in our study, we found sociality was a consistent and an important explanatory variable, positively associated with prevalence of both *Plasmodium* and *Haemoproteus*. Sociality may increase the probability of hosts encountering vectors thereby promoting parasite transmission (Ezenwa et al., 2016). It has been hypothesized that higher aggregation of vectors may occur around social species as host seeking behavior of malaria vectors relies on the odor cues (CO_2) and chemical attractants released by the host species (Lehane, 2005). This may explain higher prevalence of avian haemosporidians among social species in the Western Ghats sky island bird communities.

While there was no significant association between *Haemoproteus* prevalence and foraging strata of host species, species foraging at high strata (canopy level) exhibited lower *Plasmodium* prevalence compared to species at the ground level. Vertical stratification in arthropod vectors that influence hosts' exposure risk could drive this variation in *Plasmodium* prevalence due to differences in vector abundance. Vectors for *Plasmodium* (*Culex spp.* and *Aedes spp.*) are known to preferentially feed at the ground-level (Cerny et al., 2011; Garvin and Greiner, 2003; Mellor et al., 2000), thus reducing their abundance at the canopy level. However, our findings contrast with other studies that showed higher *Plasmodium* prevalence for middle- to high-level foragers (Astudillo et al., 2013) and low for ground foragers (Svensson-coelho et al., 2013). Although we could not yet directly assess the role of vectors in transmitting avian

haemosporidian parasites in the Western Ghats, we propose integrating information on the distribution and abundance of mosquitoes and biting midges in future research will be invaluable and help resolve these conflicting patterns.

Inter-specific variation in avian haemosporidian prevalence may also result from differences in host susceptibility to infection. Host susceptibility can vary among hosts due to host traits or differences in host-parasite coevolutionary histories (Medeiros et al., 2013). As expected, we found sexual dimorphism had a positive effect on *Plasmodium* infection risk, as has been reported in previous studies (Scheuerlein and Ricklefs 2004, Svensson-Coelho et al., 2013). This pattern of sexual dimorphism affecting haemosporidian infection lends support to Hamilton and Zuk's (1982) hypothesis whereby sexual selection favors costly male phenotypic traits (e.g., plumage brightness) as indicators of parasite resistance. Thus, species that exhibit higher levels of sexual dimorphism are likely to have higher parasite infection (Moller, 1990).

With individual body condition, we expected to find low probability of infection in birds with better condition because generally parasitic infections negatively affect host body condition (Møller et al., 1998; Sánchez et al., 2018; Schrader et al., 2003). Hosts in poor body condition are likely more susceptible to infection due to reduced immunocompetence (Beldomenico and Begon, 2010; Flint and Franson, 2009; Merrill et al., 2018). Contrary to our expectations, we found no significant association between host body condition and *Plasmodium* infection and a positive effect of body condition on the probability of *Haemoproteus* infection. Birds with better body condition had higher *Haemoproteus* infection compared to birds in poorer body condition.

Although parasites are generally thought to be detrimental to their hosts, parasites may not always be harmful to their hosts and hosts in good body condition can often tolerate higher parasite loads, leading to a positive relationship between body condition and infection status

(Budischak et al., 2018; Sánchez et al., 2018). Our findings suggest that birds were likely tolerant to *Haemoproteus* infection and did not suffer high costs to infection and or at least to the extent that it is not reflected in their body condition. However, parasitemia data and other fitness measures (e.g. reproductive success) are needed to confirm our findings of fitness costs and the underlying host defense mechanisms in response to avian haemosporidian infection in the Western Ghats. Understanding the relative investment in resistance vs tolerance is critical, as it can affect disease dynamics at both individual- and species-level. For example, highly tolerant individuals could be more efficient at transmitting disease in a population (i.e., super-spreaders; Gopinath et al., 2014). Additionally, host species that are tolerant to parasite infection may serve as reservoirs of infection and represent an indirect threat to more vulnerable host species, as has been shown for in other host-parasite systems (e.g., Adelman et al., 2013; Atkinson et al., 2013; Knutie et al., 2016), an issue critical for conservation of threatened host species.

We found higher phylogenetic signal in *Haemoproteus* compared to *Plasmodium*, highlighting a strong role of host evolutionary history in driving host susceptibility and consequently shaping patterns of parasite prevalence and disease transmission. This further suggests that host phylogeny is important for influencing variation in *Haemoproteus* infection risk, likely because closely related hosts are similar in their behavioral, physiological and immunological characteristics (Woolhouse et al., 2005) in multi-host, multi-parasite communities. This supports findings from a previous study which showed that *Haemoproteus* have high phylogenetic host specificity and tend to infect closely related host species compared to *Plasmodium*, a relatively generalist parasite (Gupta et al., 2019). Our findings that host ecological traits and host evolutionary history are both important factors in explaining the variation in prevalence of *Haemoproteus* parasites across host species suggests that constraints

on the distribution of these parasites are likely more related to their avian hosts (not vectors) within the Western Ghats sky island bird community. However, a better understanding of the relative importance of ecology of bird hosts and vectors of avian haemosporidians in the Western Ghats will be an important next step to better understand and predict patterns of infectious disease risk for these vector-borne parasites.

5. Conclusions

Taken together, our results indicate strong support for the role of ecological traits and host phylogenetic relationships in influencing variation in avian haemosporidian risk in the Western Ghats endemic bird community. As hypothesized, the relationship varies between the two avian haemosporidian genera, *Plasmodium* and *Haemoproteus*. Our analyses of various ecological factors suggest that variation in avian haemosporidian infection risk in the Western Ghats Sky Island bird community is likely driven by two underlying mechanisms. First, ecological factors (e.g. sociality, foraging strata) that may lead to differential exposure risk could impact avian haemosporidian prevalence. Second, ecological factors associated with disease susceptibility or tolerance (e.g. sexual dimorphism, body condition) to infection are important predictors of avian haemosporidian prevalence.

Interestingly, our study also revealed the importance of host phylogeny in influencing the variation of infection risk by avian haemosporidians, with higher magnitude in the case of *Haemoproteus* compared to *Plasmodium* parasites. Thus, our study highlights that patterns of avian haemosporidian prevalence and infection risk were shaped by joint contributions of both host ecology and host evolutionary history. Understanding host-parasite interactions in a broader eco-evolutionary context, including host phylogenetic relatedness is critical to gain a better understanding of drivers of variation in avian haemosporidian infection risk. Ultimately, such

efforts could help illuminate the idiosyncratic association between ecological traits and infection risk; and improve predictions of infection risk across host species, which has implications for maintaining wildlife health and conservation of threatened wildlife populations.

6. Acknowledgements

We thank the Forest Departments of Kerala, particularly then PCCFs – (R. Rajaraja Varma, V. Gopinathan, T. M. Manoharan and B. S. Corrie) and Tamil Nadu (R. Sunderaraju) for bird sampling permits. We also thank all the CCFs, Wildlife Wardens, DFOs and rangers who helped us with the logistics of field work in these remote locations. All lab work associated with this study was carried out in the lab of Uma Ramakrishnan, National Centre for Biological Sciences, Bangalore, and we thank her for the use of these facilities. Figures in the manuscript were created in Mind the Graph and Biorender platforms.

7. Funding

Financial support was provided from the U.S. Department of Energy Financial Assistance Award no. DE-EM0004391 to the University of Georgia Research Foundation. This work also was funded by Ramanujan Fellowship from Department of Science and Technology, India to GD, National Geographic Society Research and Exploration grant to V.V.R.

8. Figures

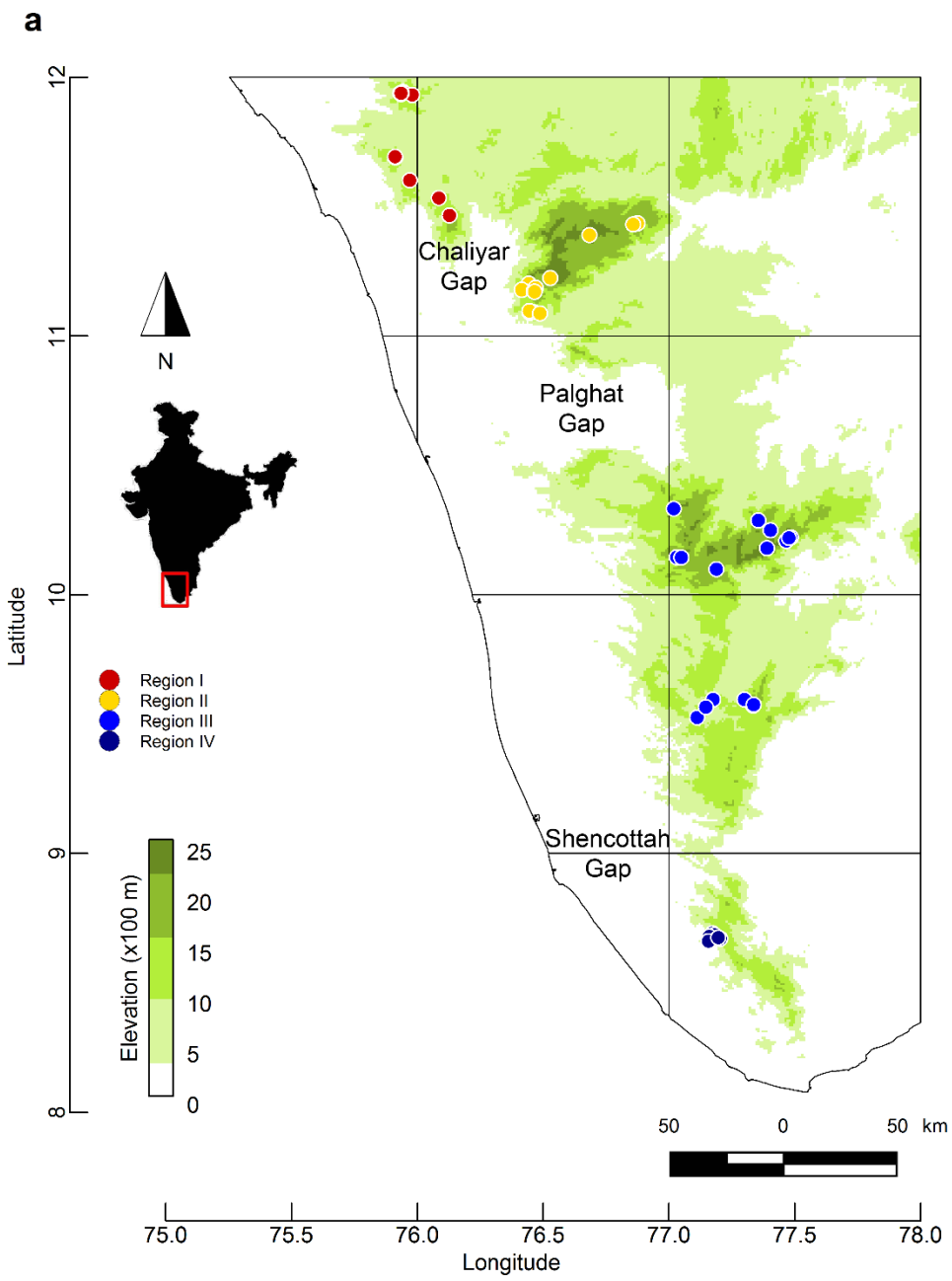


Figure 4.1. Map of Western Ghats Sky Islands including locations of sampling sites (filled circles) in four geographical regions: I (Bababudan and Banasura hills), II (Nilgiri hills), III (Anamalai-Palni-Highwavies hills), IV (Ashambu hills), corresponding to the major Sky Island group. Underlying elevation gradient in the Western Ghats is also depicted, with *Shola* Sky Islands located above 1400 m.a.s.l.

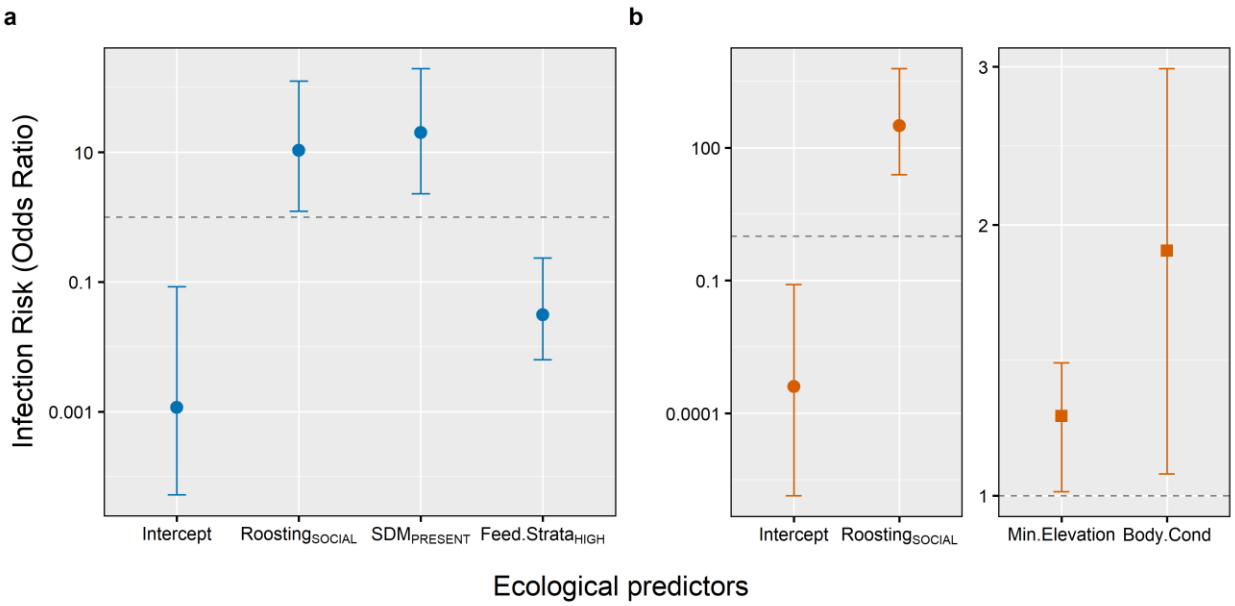


Figure 4.2. Effect of host ecological factors on avian haemosporidian infection risk in the Western Ghats Shola Sky Islands. Results of our final reduced Bayesian phylogenetic mixed model with posterior mean estimates and 95% credible intervals (CIs) of all significant predictors on infection status (*Plasmodium*, blue and *Haemoproteus*, orange). Model parameters were considered as significant when the 95% CIs of posterior estimates excluded zero. Categorical variables tested include Roosting behavior (non-social *vs*. social), Sexual dimorphism (absent *vs*. present), Feeding strata (low *vs*. high), with the former as the reference category and two covariates: Species minimum elevation and individual body condition (scaled mass index).

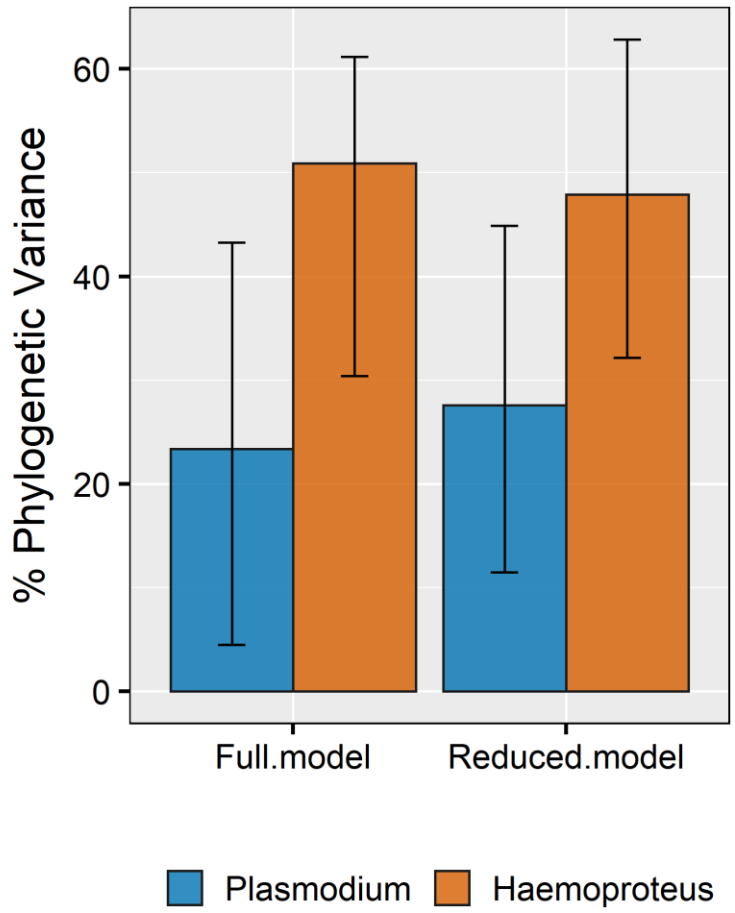


Figure 4.3. Proportion of total variance attributed to host species phylogeny representing phylogenetic signal or lambda (k). Reported are the percent posterior means and 95% credible intervals across full and reduced Bayesian phylogenetic mixed models estimated in MCMCglmm, shown for *Plasmodium* (blue) and *Haemoproteus* (orange).

CHAPTER 5

TARGET SEQUENCE CAPTURE FOR PHYLOGENOMIC RESEARCH OF DIVERSE HAEMOSPORIDIAN PARASITES INFECTING MAMMAL AND BIRD HOST SPECIES[4]

[4] **Gupta, P.**, Faircloth, B.C., Glenn, T.C., & Dharmarajan, G. Target sequence capture for phylogenomic research of diverse haemosporidian parasites infecting mammal and bird host species. To be submitted to *Molecular Ecology*

1. Introduction

Haemosporidians (Phylum: Apicomplexa, Order: Haemosporida) are a diverse group of vector borne protozoan parasites that infect numerous vertebrate hosts, including mammals, birds and reptiles (Garnham, 1966; Perkins, 2014a; Telford, 2009; Valkiŭnas, 2005). Most common haemosporidian genera include *Plasmodium* (mammal and bird hosts), *Haemoproteus* and *Leucocytozoon* (bird hosts), *Hepatocystis* (bat hosts), among others. Haemosporidians are transmitted via dipteran vectors such as mosquitoes (Culicinae), biting midges (Ceratopogonidae), louse flies (Hippoboscidae) and simuliid flies (Simuliidae). About 300 species of haemosporidians have been described across 20 haemosporidian genera, with over 200 species belonging to just one genus: *Plasmodium* (Bensch et al., 2009). Due to its diversity and role as a causative agent for human malaria, numerous studies have focused on *Plasmodium* species infecting primates and rodents, and consequently, large-scale genomic data are available primarily from these parasites. For instance, complete genomes are available for primate malaria parasites— *P. falciparum* (Gardner et al., 2002), *P. vivax* (Carlton et al., 2008), *P. ovale* and *P. malariae* (Rutledge et al., 2017), *P. reichenowi* (Otto et al., 2014b), *P. knowlesi* (Pain et al., 2008), *P. cynomolgi* (Tachibana et al., 2012) and the three rodent malaria parasites— *P. yoelii*, *P. chabaudi* and *P. berghei* (Carlton et al., 2002; Hall et al., 2005; Otto et al., 2014a). However, other haemosporidian parasites such as those infecting birds, which have contributed to our early understanding of human malaria parasites and advanced our understanding of ecological and evolutionary dynamics of infectious diseases, in general, have received less attention (Videvall, 2019).

In the last few decades, molecular sequence data has increasingly been employed to resolve the haemosporidian phylogeny and investigate its evolutionary origins but lack consensus. The first molecular study (based on 18S ribosomal DNA) examining the evolutionary origins of mammalian malaria parasites concluded that *P. falciparum* was a sister species to avian malaria parasite and originated as a result of host switch from chickens to humans (Waters et al., 1991). However, contemporary studies with a broader taxon sampling and mitochondrial gene phylogeny found support for the paraphyletic relationship of *Plasmodium* parasites, with *Haemoproteus* and *Hepatocystis* (Perkins and Schall, 2002) nested within the *Plasmodium* clade. A multigene (nuclear, mitochondrial and apicoplast gene) phylogenetic analysis by Martinsen et al. (2008) showed that mammalian *Plasmodium* parasites along with *Hepatocystis* parasites infecting bats form a separate clade from *Plasmodium* infecting birds and lizards. They also showed that avian genus *Haemoproteus* is polyphyletic with two subgenera *Haemoproteus* and *Parahaemoproteus*; *Leucocytozoon* is sister to all other genera. Furthermore, phylogenetic analysis by Borner et al. (2015) based on multiple nuclear genes (21 genes) recovered *Plasmodium* as a monophyletic taxon, including bird *Plasmodium* and *Leucocytozoon* as a basal taxon group. The authors also recovered *Polychromophilus* as a sister clade to the genus *Plasmodium.* However, Galen et al. (2018) recently proposed the most comprehensive phylogeny of malaria parasites based on multi-locus dataset (21 nuclear genes) and included diverse Haemosporidian parasites. They recovered polyphyly of genus Plasmodium, with genera *Hepatocystis* and *Nycteria* nested within the *Plasmodium* clade that infects primates, rodents, bats, lizards and birds; *Plasmodium* parasites infecting ungulates were recovered as a sister clade to *Polychromophilus.* Additionally, *Leucocytozoon, Haemoproteus* and *Parahaemoproteus* were

found as a sister taxon group to the rest of the haemosporidian parasite clade. Overall, several phylogenetic hypotheses have been tested for deciphering the evolutionary history of haemosporidian parasites but are still inconsistent due to variable taxon sampling and type of molecular data used. Thus, a diverse taxon sampling and genomic-level data are needed for a robust comparative phylogenetic analysis and tracing multiple host switches that may have occurred in the order Haemosporida.

In addition to lack of well resolved phylogenies for haemosporidian parasites in general, the taxonomy and evolutionary origins of avian haemosporidians, in particular, remains unresolved (Carlton et al., 2013; Perkins, 2014b). There is a huge gap in our understanding of relationship between avian malaria parasites and other malaria parasites, due to the lack of genomic data and limited number of informative molecular markers. Avian haemosporidians have been widely used as a natural system for understanding ecological and evolutionary dynamics of host-parasite interactions but a well-resolved phylogeny is critical for making robust phylogenetic inferences. Sequencing of parasite mitochondrial *cytb* gene ~478bp is the most common molecular method for examining parasite prevalence, genetic diversity, host susceptibility and evolutionary dynamics of avian haemosporidians. Though fast and relatively sensitive compared to microscopy, evolutionary studies based on a single genetic marker has some limitations (e.g. presence of cryptic diversity, detecting mixed infections and lack of well resolved phylogenies).

Understanding patterns of host switching among the malaria parasites is important from the perspective of emerging infectious disease as some genus (e.g., *Plasmodium*) hold a greater emergence potential in naïve host communities, which do not share a long co-evolutionary

history with the parasite. Consequently, *Plasmodium* spp. have severely affected wild bird populations in Hawaii and New Zealand island (Alley et al., 2010; Atkinson et al., 2000; Schoener et al., 2014; Warner, 1968). It is therefore critical to understand the molecular mechanisms underlying this increased virulence of *Plasmodium* parasites compared to *Haemoproteus* or *Leucocytozoon*, which are generally benign in natural populations. In this regard, genomic tools can help us understand the functional underpinnings of infectious diseases at a much higher resolution compared to conventional molecular approaches. Recently, draft genomes of *P. relictum* (DONANA05), *P. gallinaceum* 8A and *H. tartakovskyi* became available, along with a transcriptome of *Plasmodium ashfordi* (GRW2) (Bensch et al., 2016a; Böhme et al., 2018; Videvall et al., 2017). Apart from genomic data, blood transcriptomes of four avian *Plasmodium* spp. (*P. ashfordi*; Videvall et al., 2017), one *Haemoproteus* and a partially sequenced transcriptome for *Leucocytozoon buteonis* has been described but the latter is not yet filtered from host contigs (Pauli et al., 2015). However, most of the genome and transcriptome data for avian haemosporidians has been obtained from experimentally infected hosts and obtaining high-quality genomic sequence data from wild bird samples has proven to be quite difficult (but see Galen et al., 2019).

There are several challenges that have hindered acquisition of genomic data from avian haemosporidians. First, the presence of nucleated erythrocytes in bird hosts disproportionally increases host DNA compared to parasite DNA and obtaining adequate quantities of parasite DNA for high-throughput sequencing becomes difficult. Second, the intensity of parasite infection (parasitemia levels) in wild bird populations is very low; generally parasites infect 0.0001 to 0.1% of the red blood cells which further reduces the amount of parasite DNA

obtained from field samples (Zehtindjiev et al., 2008). Third, host and parasite genome sizes differ substantially, with *Plasmodium spp.* genome approximately 2.5 Mb and the avian genome ~0.9-2.2 Gb (Carlton et al., 2013; Kapusta et al., 2017). Fourth, high-throughput sequencing is further complicated by extreme AT-content of avian haemosporidian genomes, with avian *Plasmodium* having a GC-content of ~21%, making it the most AT-biased genome of all eukaryotes sequenced to date (Videvall, 2018).

In the last few years, two innovative methods have been developed to overcome the challenge of obtaining pure parasite DNA but have some limitations. First, Palinauskas et al. (2013) developed a method to isolate purified genomic DNA based on in vitro manipulation of *Haemoproteus* gametocytes to undergo exflagellation, a process that naturally occurs when a vector ingests bird blood. The resulting microgametes can then be separated from the host cells by centrifugation. One of the limitations of this method is that parasitemia in infected hosts must be ≥2%; in addition, it cannot be used to isolate pure genomic DNA from *Plasmodium* spp. Collection of microgametes in *Plasmodium* spp., unlike *Haemoproteus* spp., is not straightforward and requires additional stimuli (e.g. xanthurenic acid) for initiating the process of exflagellation (Arai et al., 2001). Second, Lutz et al. (2016) used laser capture microdissection microscopy (LCMM) to isolate parasite DNA. The authors extracted individual parasite cells directly from the hosts' nucleated cells and performed whole genome amplification prior to sequencing. However, the isolation of individual parasites will not be a feasible technique in a field setting or when large number of samples need to be processed to test phylogeographic hypotheses.

To address these challenges (described above), recent advances in high throughput sequencing methods such as target sequence capture offer an excellent alternative to increasing the ratio of parasite vs. host DNA and has shown great potential in obtaining genomic data from avian malaria parasites. For example, sequence capture approaches allows simultaneous capture and sequencing of hundreds to thousands of loci from mixed species samples or parasite infected host cells (Faircloth et al. 2012; Lemmon et al. 2012; McCormack et al. 2013; Glenn and Faircloth 2016; Jones and Good 2016). It enables capture of genomic data by using baits designed from the species of interest (when a reference genome is available) or a distantly related species (when a reference genome is not available) and thus can encompass a broad range of taxa.

Here, we developed UCE probes targeting conserved loci across all available *Plasmodium* and *Haemoproteus* genomes and used sequence capture approach and next generation sequencing to characterize the genome of avian haemosporidians and resolve its evolutionary relationships with other mammalian malaria parasites. Obtaining genomic data from a diverse group of haemosporidians will aid in comparative genomics, assess evolutionary mechanisms underlying diversification of haemosporidians (e.g. host switching), molecular mechanisms underlying host-parasite interactions, and provide further insights into evolutionary dynamics of malaria parasites. It will also help to generate a large suite of universal markers that can be employed across haemosporidians and help to resolve evolutionary relationships over both deep and shallow evolutionary timescales. Moreover, the ability to generate genomic data from chronically infected wild bird samples (low parasitemia) will open prospects for further

exciting research on the evolutionary epidemiology of avian malaria parasites and test other broad phylogenetic and phylogeographic hypotheses.

In this study, our goal was to a) identify malaria-UCE baits useful for inferring evolutionary relationships across a wide diversity of *Plasmodium* and *Haemoproteus spp*., b) optimize and evaluate the sensitivity of target sequence capture and next generation sequencing for obtaining good quality genomic data from haemosporidians sampled from wild birds, and c) examine phylogenetic relationships among haemosporidians.

2. Methods

2.1. Probe Design

We designed UCE baits (capture probes, ~160 bp) following the standard UCE workflow as described in Faircloth, (2016). We chose four representative *Plasmodium* genomes (*P. chaubadi*, *P. falciparum*, *P. vivax* and *P. gallinaceum*) and one *Haemoproteus* genome (*H. tartakovskyi*) based on their clade-level relationships across the Haemosporidian phylogeny (Bensch et al., 2016b; Böhme et al., 2018; Lutz et al., 2016b) to design target capture probes. We used broad taxonomic range of *Plasmodium* and related haemosporidians to develop a diverse set of probes that would allow us to study deep evolutionary relationships among haemosporidians. Briefly, the genome assemblies of three *Plasmodium* and one *Haemoproteus* (species as mentioned above) were retrieved from NCBI and aligned against the reference/base genome sequence of *P. gallinaceum* (avian *Plasmodium*) using a permissive read aligner. The unmapped reads were removed, and mapped reads were further filtered to remove repetitive regions to get a diverse set of 3070 bait sequences, targeting 396 loci that were conserved and shared among all the *Plasmodium* and *Haemoproteus* species. Initial *in silico* analysis resulted in recovery of 380

loci across the *Plasmodium* and *Haemoproteus* genomes; including five haemosporidian genomes used for generating baitset and other *Plasmodium* genomes of interest (*P. ashfordi, P. relictum, P. berghei, P. yoelii, P. knowlesi, P. ovale, P. malaria* and *P. reichenowi*). The bait sequences were then sent for synthesis in a custom MYbaits kit by MYcroarray (now Arbor Biosciences, Ann Arbor, MI).

2.2. Genomic Sample Preparation

We evaluated the effectiveness of sequence capture approach in obtaining genomic data from avian and other related haemosporidians in two experiments with laboratory and field acquired parasite samples. In our first preliminary experiment, we tested clinical parasite strains acquired from various sources and included reference strains for *P. falciparum, P. berghei, P. chabaudi, P. yoelii, P. knowlesi, P. cynomolgi, P. gallinaceum* and *P. relictum.* After the initial testing, we aimed at obtaining genomic data from field-collected bird samples. We obtained 22 genomic DNA samples from naturally infected bird hosts that had been found to be infected with avian haemosporidians (based on microscopy and PCR screening) in MY laboratory (details in Appendix C, S5.1). We also included clinical strains of *P. gallinaceum* and *P. relictum* as controls along with the field samples, giving us a total of 24 samples in our second experiment. Individual libraries were prepared for each of these samples, following the standard protocol as outlined below, but pooled libraries from both experiments were analyzed in separate sequencing runs.

2.3. Library Preparation

We quantified DNA concentration of each sample using a QUBIT 3.0 Fluorometer and fragmented 100-500 ng them using a Covaris ultrasonicator (Covaris, Inc.) to obtain an average

fragment size distribution of ~ 400 to ~ 500 bp. The sonication step was conducted with the following parameters: 3 min (with 10 s pulse on, and 10 s pulse off), and the amplitude set at 20%. All sheared samples were checked on a 2% agarose gel to ensure that genomic DNA was sheared at approximately the selected fragment size. The fragmented DNA samples were end-repaired and A-tailed using the KAPA Hyper Prep Kit (Kapa Biosystems), following the standard protocol suggested by the manufacturer, except we used half-volume reaction sizes throughout. We also prepared eight additional libraries with randomly chosen field samples, using the NEBNext Ultra II DNA Library Prep kit for Illumina (New England Biolabs, MA, USA) in order to test whether sequencing results were affected by differences in library kits generally used for sequence capture. Each library was barcoded using unique dual-indexed i5-i7 adapters (Glenn et al., 2019) and 14 cycles of indexing PCR were performed to amplify the adapter–ligated DNA fragments. Post-ligation cleanup was performed using 1:1 SPRI-substitute reagent, which was also used in all cleanup steps.

2.4. Capture and Sequencing

Library quantities were checked using the Qubit Fluorometer and then pooled in groups of eight samples, in equimolar ratios to yield 500 ng of total library per capture pool, prior to hybiridization. Pools were evaporated in a speed vacuum centrifuge, and then were resuspended in 7 µl of dH_20. For the first experiment including clinical strains, we retained a part of the same pooled library to be sent for direct sequencing without targeted sequence capture (pre-capture, i.e., an unenriched library). We also diluted a part of this pooled library with chicken DNA (1:1000) and followed with sequence capture (hereby referred to as diluted-capture) to assess the sensitivity of malaria UCE probes, giving us a total of three pooled libraries in our first

experiment. For sequence capture, we used the custom MyBaits kit (MYcroarray, Ann Arbor, MI, USA) designed for malaria parasites, and followed the manufacturers protocol, but replaced the kit's blocking agent (human Cot-1 DNA) with chicken Cot-1 DNA (Applied Genetics Laboratory). After enrichment, we conducted a 16-cycle PCR reaction on the captured libraries using KAPA HiFi HotStart ReadyMix (Kapa Biosystems, USA) and Illumina primer mix and quantified enriched libraries using a QUBIT 3.0 Fluorometer.

In total, two pools from the first experiment and four pools from the second experiment containing target-enriched libraries were sent for 150 bp paired-end sequencing on an Illumina HiSeq 2000 (Illumina, USA). As some samples from the second experiment showed low yield (low number of raw reads) in our first run, we re-enriched three previously enriched library pools (hereby referred as double capture), following the same protocol as described above. The re-enriched libraries were then sequenced using 150 bp, paired-end sequencing on an Illumina HiSeq 2000 (Illumina, USA).

2.5. Raw Read Processing

The three datasets (from experiment 1, 2 and double captures) were analyzed together and processed using the PHYLUCE pipeline (ver. 1.6.6; Faircloth, 2016) The bioinformatics workflow is also outlined in figure 5.1. Demultiplexed raw sequence reads were checked for quality in FastQC (ver. 0.8.11; Andrews, 2015). We trimmed raw FASTQ data for removal of adapter sequences and low-quality bases using Ilumiprocessor, a parallel wrapper program which incorporates Trimmomatic (ver. 0.36; Bolger et al., 2014). We set a quality cut-off of 25 over a 4-bp sliding window, discarding any trimmed read shorter than 40 bp. We evaluated cleaned,

trimmed reads in FastQC to ensure that only high-quality reads were retained, and adaptors were completely removed.

2.6. Extracting target enrichment data

Although, our sequence capture approach targeted UCE loci in each sample library, the abundance of off target reads will be substantially higher due to host contamination, thus making *de novo* assemblies computationally challenging. Thus, we first performed read mapping to filter out the reads that mapped only to parasite genomes, increasing our chances of recovering DNA of targeted loci. Filtered and adapter cleaned reads were mapped to *Plasmodium* and *Haemoproteus* reference genome sequences retrieved from GenBank, using bwa-mem (default parameter settings) and duplicates were marked using Picard's markduplicates. QualiMap was used to generate mapping statistics from the BWA mapping output. We then used SAMTOOLS to retrieve the mapped reads from the BAM alignment files and convert them back to fastq reads. Subsequently, mapped fastq reads were *de novo* assembled into contigs with Abyss v.1.9.0 (Simpson et al., 2009) , specifying a predefined k-mer length, k = 55. The resultant contigs were then matched against target probe sequences using LASTZ with the python script 'assembly_match_contigs_to_probes.py' to identify individual UCE loci. Contigs that were 65% identical and covered 65% of the total length of the target probe sequences were retained. We also discarded any potential paralogs, including contigs that match multiple UCE loci, or different contigs that match the same UCE locus. After UCE locus identification, we extracted FASTA sequences for all enriched UCE loci recovered from a minimum of three samples by using 'get_match_counts.py' and 'get_fastas_from_match_counts.py' python scripts. This resulted in an incomplete matrix as some loci may be missing from some taxa.

2.7. Sequence alignment, trimming and phylogenetics

We aligned FASTA sequences for each UCE locus obtained at the preceding step using 'phyluce_align_seqcap_align' which implements MAFFT (ver. 7.130b; Katoh and Standley, 2013). We trimmed alignments with GBLOCKS (ver. 0.91b; Castresana, 2000; Talavera and Castresana, 2007), using the script 'phyluce_align_get_gblocks_trimmed_alignments_ from_untrimmed' and default settings implemented in PHYLUCE. Finally, we selected 65%, 70% and 90% data matrix completeness per locus (i.e., the proportion of taxa represented in each given locus) using the script 'phyluce_align_get_only_loci_with_min_taxa' and generated a concatenated matrix using the script 'phyluce_align_format_nexus_files_for_ raxml' for subsequent phylogenetic analysis. We built phylogenetic trees using 60%, 75% and 90% data matrices by first conducting 20 maximum likelihood (ML) search for best-fitting tree using the GTRGAMMA substitution model in raxmlHPC-MPI-SSE3 v8.2 (Stamatakis, 2014). We then performed non-parametric bootstrap replicates using the autoMRE option to optimize the number of bootstrap replicates for the dataset and finally reconciled the best fitting ML tree with the bootstrap replicates.

3. Results

In our first experiment, for the pre-capture data, the number of reads per sample ranged from 143,100 to 469,000 with an average of 300,413 paired end reads across samples (Appendix C, S5.2). In the diluted-capture data, the number of reads per sample ranged from 400 to 33,200 with an average number of 8,471 paired end reads. In post-capture data, the number of reads per sample ranged from 9,500 to 1,077,100 with an average number of 273,025 paired end reads across samples. Raw reads have been deposited in the National Center for Biotechnology

Information (NCBI) Sequence Read Archive (BioProject #). Targeted sequence capture resulted in considerable improvements in the number and percentage of parasite reads (i.e., reads that matched to respective parasite genomes using bwa), with ~ 2- to 9-fold increase in the percentage of parasite reads in post-capture vs pre-capture libraries (figure 5.2a, Appendix C, S5.3). Overall, we did not find any overwhelming trend in fold enrichment for each species or the phylogenetic distance from the species used in bait design, except for *P. falciparum*, which showed no fold enrichment. This could be explained by the starting quality of genomic DNA as they were pure parasite samples and thus contained substantial number of parasite reads even in unenriched libraries. The total number of UCE loci recovered were similar or better in post-capture vs diluted post-capture libraries but substantially higher compared to pre-capture libraries, which supports our expectations (figure 5.2b, Appendix C, S5.3).

In the second experiment, we obtained an average of 978,093 paired end reads (range 14,400 to 5,063,000 reads) in our single capture run and 1,754,934 average number of paired end reads (range 1,277 to 12,143,787 reads) in the double capture run (Appendix C, S5.2). Bwa mapping results revealed a higher percentage of reads that matched to *Plasmodium* and *Haemoproteus* reference parasite genomes from the double captured samples than single captured samples, with an average of 15.67% reads (range 4.60 to 42.90%) and 28.70% reads (range 0.5 to 60.10%), suggesting that double captures substantially improved the proportion of parasite reads (figure 5.3a). The mapped reads were assembled into an average of 4911 contigs (range 579 to 26520 contigs) per sample for single captures and an average of 9757 contigs (range 11 to 80048 contigs) for the double capture data (Appendix C, S5.2).

Across the single captures, we successfully recovered > 100 UCE target loci (up to 232 loci) from 8 out of 15 samples. For the double capture data, we obtained > 100 UCE target loci (up to 223 loci) from 10 out of 18 samples (figure 5.3b), excluding samples from one of the pools (Pool 3) which showed recovery of fewer than 30 UCE loci (Appendix C, S5.2). Although the number of UCE loci extracted was lower for some samples across both single and double captures, there was no obvious phylogenetic bias. Less locus recovery for some samples also occurred as a result of UCE loci that were lost during data processing and were discarded after the validation step in PHYLUCE where multiple contigs that matched to single UCE loci are removed. In total, across both the experiments, 22 samples were sequence capture successes, with more than 100 loci recovered out of 396 target UCE loci. Details on UCE loci extracted from each sample across runs and summary statistics are given in Appendix C, S5.2. For phylogenetic analyses, our final alignment data included 22 samples from all runs;18 samples with 100 UCE loci to avoid too much missing data and 4 samples that had <50 loci. Final number of loci in the concatenated dataset contained 16 loci (90% completeness), 121 loci (75% completeness) and 192 loci (60% completeness), with a mean length of 202 bp (range 81 bp to 496 bp). Phylogenetic relationships across major groups were quite similar with 90% complete data matrix and 75% complete data matrix. Thus, phylogenetic tree with 75% complete data matrix is shown here (figure 5.4). Based on our preliminary phylogenetic analysis, as expected, we found all mammalian malaria parasites to group together in a separate clade than bird malaria parasites (figure 5.4). Surprisingly, bird *Plasmodium* and *Haemoproteus* parasites did not form two separate clades, as has been observed in previous studies (Galen et al., 2018). But earlier studies have primarily used either mitochondrial data or data based on few nuclear genes and the

use of genome-level data as shown here potentially challenges the known evolutionary relationships among avian haemosporidians at the genus level. However, it is important to note that this is merely a snapshot of the dataset generated in this study and more robust phylogenetic analysis such as building a Bayesian phylogenetic tree are needed to confirm these findings. We hope that further phylogenetic analysis using the full dataset and more advanced phylogenetic tools will provide a more comprehensive picture and shed further insights into this unique phylogenetic relationship observed among avian haemosporidians.

4. Discussion and Conclusion

In this study, we designed and tested the utility of a novel malaria-UCE bait set using sequence capture and high throughput sequencing for obtaining genome level data in all major groups of avian and mammalian malaria parasites. Our results show that sequence capture approach successfully allows acquisition of parasite DNA from mixed DNA samples when host contamination is disproportionately higher than parasite DNA and provides further resolution of phylogenetic relationships among diverse group of haemosporidian parasites. Overall, we obtained good number of sequencing reads and UCE loci recovery for each sample, and across pools, except for Pool 3, which showed low number of UCE loci, despite a good starting number of raw reads after sequencing. The low success rate for these samples could likely be explained by either low parasitemia levels or issues associated with these pooled libraries during hybridization of capture probes.

Target sequence capture approach based on highly conserved UCE loci, resulted in haemosporidian parasite genomic sequences with almost equal probability of success across diverse clinical and field parasite strains, with success capture up to 25% –60% UCE loci. We

found improved recovery of UCE loci from some samples (e.g. samples that contained fewer UCE loci in single capture) after double captures while others gave similar number of UCE loci in single vs double capture. This suggests that performing double captures might be an effective strategy to get consistent and reliable parasite data, especially for field collected bird samples as they often have low parasitemia. We did not observe any obvious phylogenetic bias in number of loci recovered across samples, which is likely because of the design of malaria-UCE probe set as it included probes from genomes of all major haemosporidian genera. The sequence capture approach described here is especially advantageous as it offers potential to obtain genomic data from other malaria genome, e.g., bat and ungulate malaria parasites that still lack genomic data. Obtaining genomic data based on a set of more conserved loci across diverse haemosporidian taxa in conjunction with phylogenomic and population genomic approaches will improve our ability to resolve both deep and shallow phylogenetic relationships, applicable for a wide range of haemosporidians.

Previous studies using target capture for avian haemosporidians have been limited in scope. Recently, targeted enrichment approach was used to obtain exon-capture data from naturally infected bird samples (Barrow et al., 2018; Huang et al., 2018). Although the method was successful, it was limited in scope as baits developed were based on newly sequenced *H. tartakovskyi* genome and focused primarily upon the exome. The authors reported that locus recovery declined with increasing levels of divergence from the reference genome. Some recent studies have used transcriptome sequencing (e.g., RNA-seq; Galen et al., 2019) as another potential approach for obtaining genomic data across many loci for phylogenetic analysis. However, obtaining fresh RNA is difficult or often not feasible in field settings and cannot be

used for bird samples that have already been collected by researchers. Thus, sequence capture approach based on genomic DNA offers a more promising avenue to advance avian haemosporidian research and improve our understanding of ecological and evolutionary processes driving disease dynamics.

5. Figures

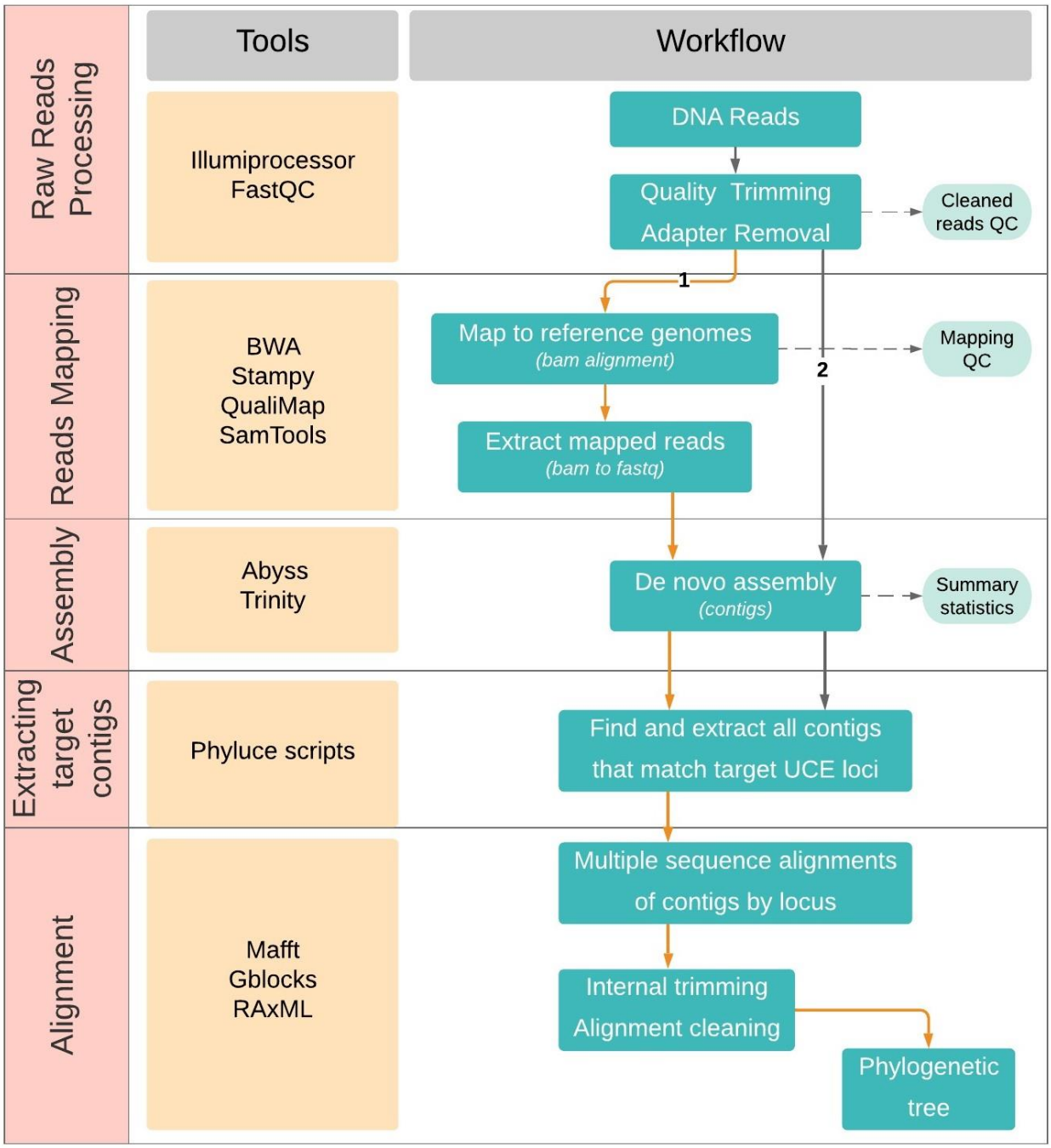
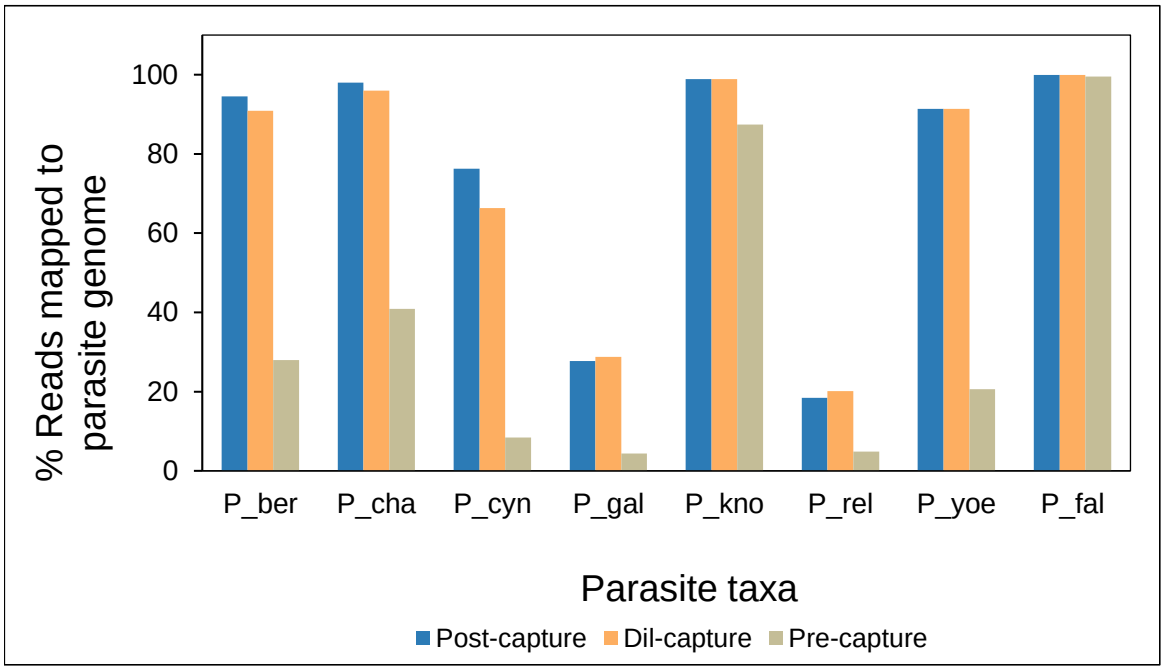


Figure 5.1. UCE phylogenomics workflow used for bioinformatics processing of target enrichment data obtained from apicomplexan haemosporidian parasites.

a)



b)

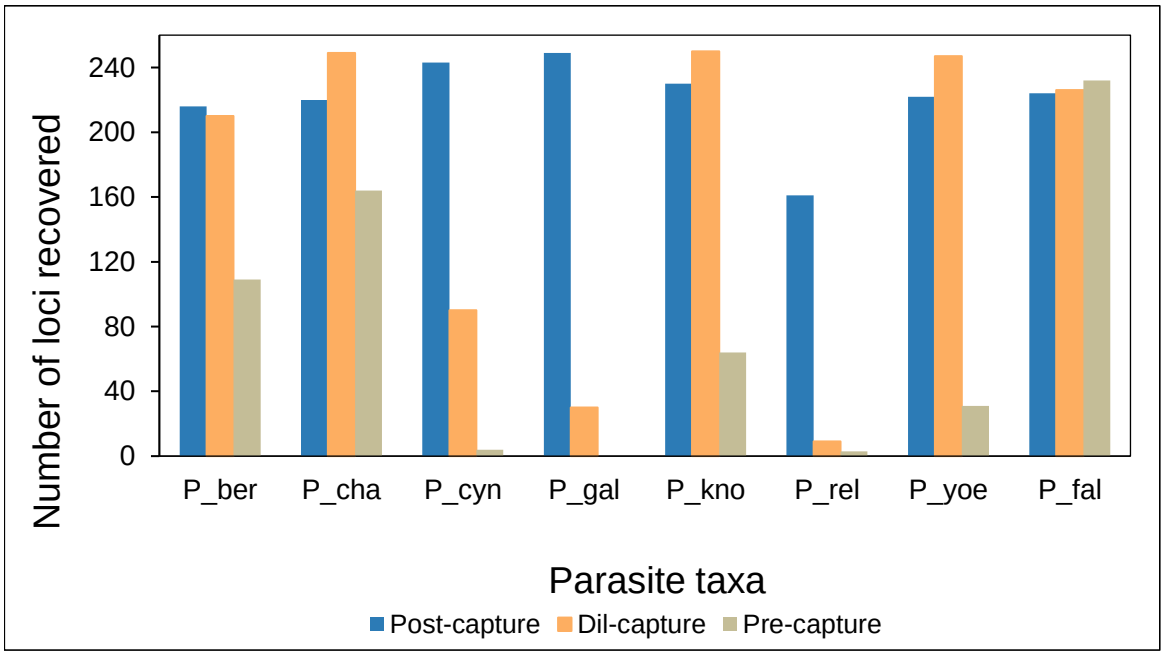
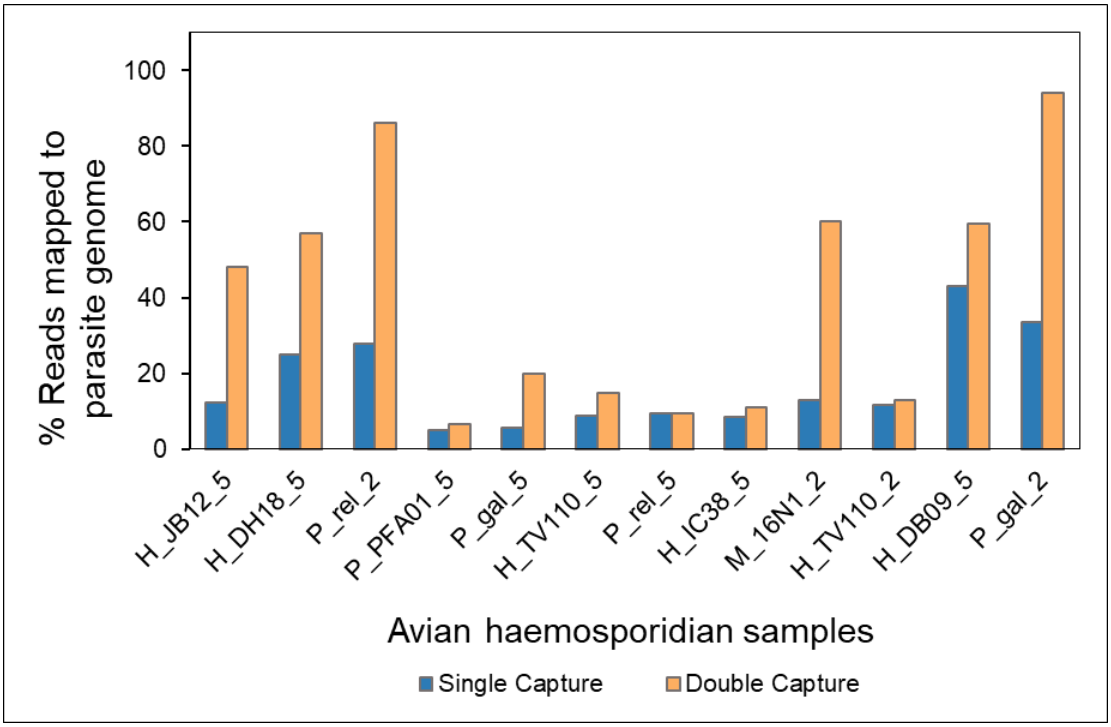


Figure 5.2. Comparison between post-capture, diluted-capture and pre-capture libraries, in terms of (a) percentage of total reads mapped to parasite genomes and (b) number of UCE loci recovered in contigs assembled from mapped reads.

a)



b)

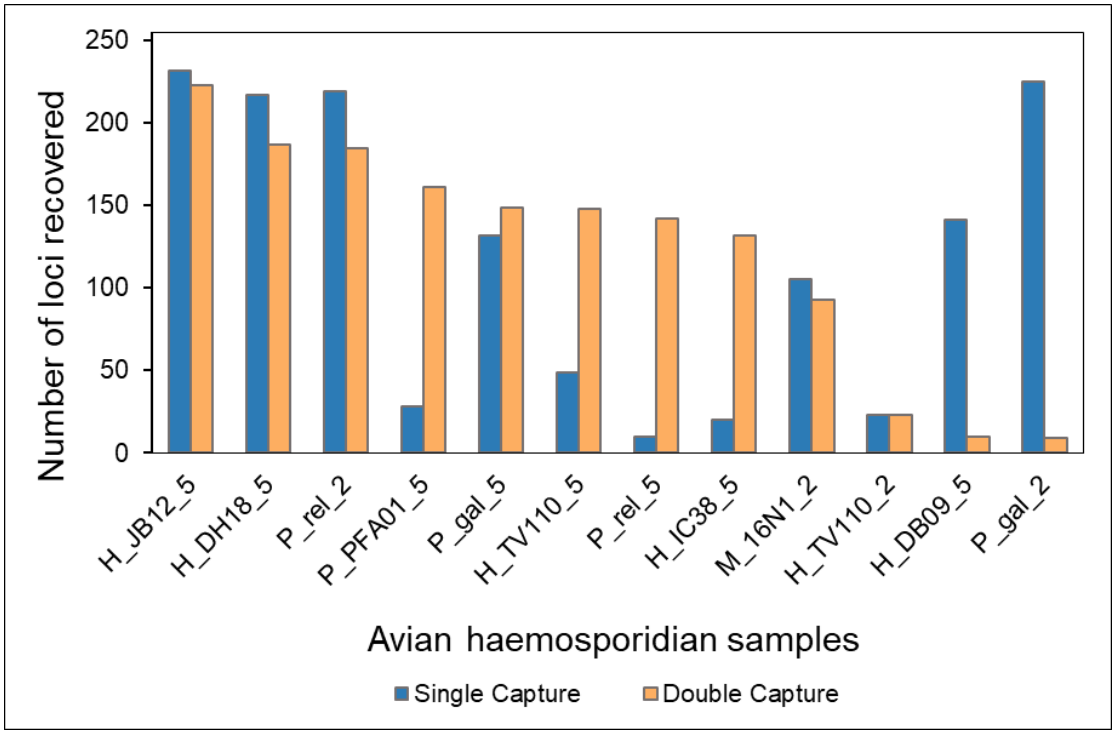


Figure 5.3. Comparison between single and double captures, in terms of (a) percentage of total reads mapped to their parasite genomes and (b) number of UCE loci recovered.

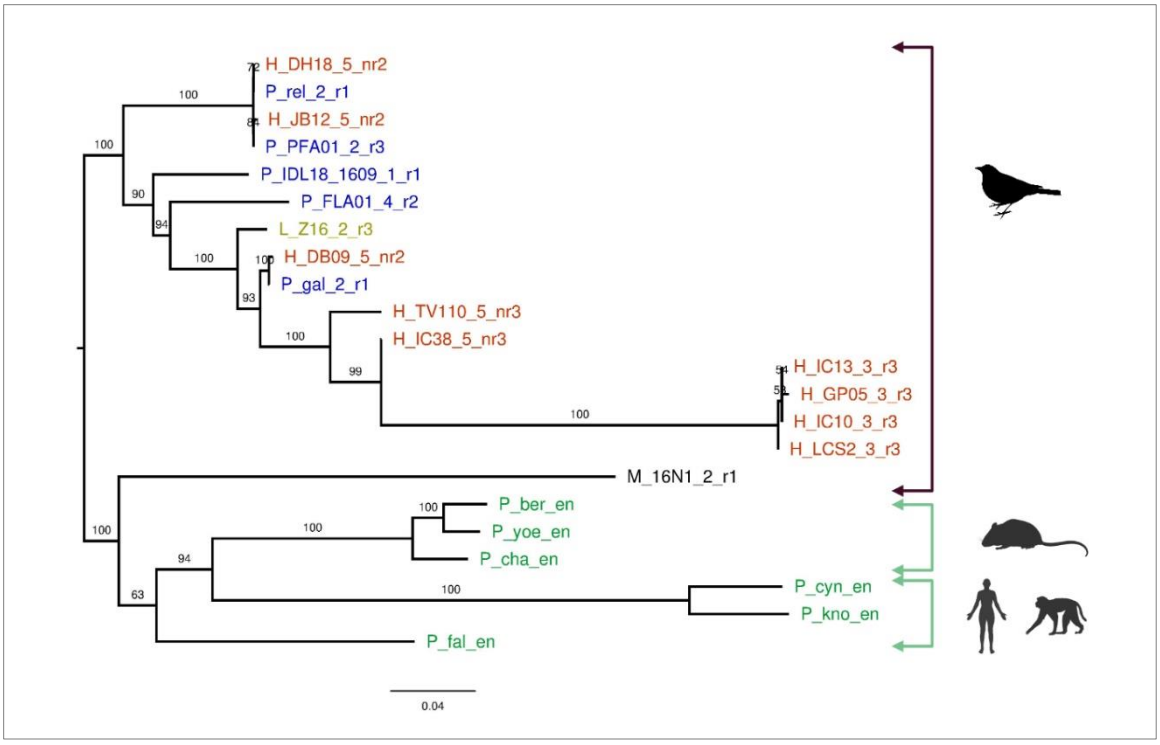


Figure 5.4. Phylogenetic relationship of avian haemosporidians and other related malaria derived from genome-scale data. Shown here is the best fitting ML tree with bootstrap support as branch labels and tree tip labels are the sample IDs (with parasite genus (P/H/L), ID, pool and run no. separated by underscores). The tree was based on concatenated dataset (75% complete data matrix) with 121 UCE loci.

CHAPTER 6

CONCLUSIONS

Emerging infectious diseases have become increasingly common over the last several decades and are one of the major challenges of our times threatening both human and wildlife health. Global biodiversity has been affected by and will likely suffer the devastating effects of the rapid increase of these new and existing infectious diseases. While we are generally more concerned about infectious diseases affecting human health, influence of emerging infectious diseases on wildlife health and its cascading effects on ecosystem health are less well recognized but extremely important. Indeed, about 70% of infectious diseases that have emerged in humans in the last few decades are zoonotic, including the current COVID-19 global pandemic. Consequently, it is critical to elucidate the ecological and evolutionary drivers of infectious diseases to better understand and manage emerging infectious diseases in human and wildlife populations. Building on this conceptual framework, my dissertation research investigated ecological and evolutionary dynamics of infectious diseases using avian haemosporidian parasites as a model system. Specifically, I was interested in: i) exploring whether geographical and host factors drive emergence of some parasites vs. others in novel host communities, ii) understanding whether host ecological traits and evolutionary history influences the risk of infection among host species, iii) developing genomic tools to better understand evolutionary history of avian haemosporidians and further advance comparative phylogenomics of malaria parasites.

My dissertation research presented a first comprehensive investigation of the prevalence, diversity, and community structure of avian haemosporidians in the Western Ghats, an important global biodiversity hotspot. By sampling almost the entire Shola Sky Island bird community, I found critical ecological and evolutionary differences between *Plasmodium* and *Haemoproteus*, which are related group of avian haemosporidians (Chapter 3). *Plasmodium* parasites were host generalists, infecting multiple bird species whereas *Haemoproteus* were primarily host specialists, restricted to one or few closely related host species. *Haemoproteus* parasites also revealed strong signal of co-speciation with their hosts, suggesting it as one of the dominant mechanisms of co-evolutionary diversification compared to host-switching. This is likely because of the old origin of the Western Ghats mountain range with many endemic host radiations and long co-evolutionary history of avian hosts with their haemosporidian parasites. One of the most surprising findings was that despite the strong co-phylogenetic associations between *Haemoproteus* parasites and their hosts, genetic structure of *Haemoproteus* parasites was not affected by biogeographic barriers. Host species (e.g. *Sholicola* spp. and *Montecincla* spp.) that genetically diverged approx. 4-5 Ma due to the biogeographic gaps carried similar *Haemoproteus* lineages across their range. This pattern could likely be due to differences in mutation rates of *Haemoproteus* parasites vs. their hosts, possible role of dipteran vectors in facilitating dispersal of *Haemoproteus* lineages across the gaps and/or the lack of power of partial mitochondrial loci to reveal fine-scale population structure of *Haemoproteus* parasites. Using genomic-level data for avian haemosporidian parasites could provide better insights into the phylogeography of *Haemoproteus* parasites and is an exciting, open research direction. Finally, as expected, results from the global phylogenetic analyses of avian haemosporidian

parasites revealed that generalist *Plasmodium* parasites from the Western Ghats were widely interspersed whereas specialist *Haemoproteus* lineages were phylogenetically more clustered, supporting the increased emergence risk of *Plasmodium* parasites. Overall, this study (Chapter 3) provided significant insights into understanding why some parasites (e.g. *Plasmodium*) rapidly emerge in novel communities while others do not (e.g. *Haemoproteus*).

Investigations into variation in infection risk across Shola Sky Island bird communities revealed that host ecological traits and host phylogeny jointly determine why some species have higher infection risk compared to others (Chapter 4). This study showed that host social behavior was an important predictor associated with both *Plasmodium* and *Haemoproteus* infection risk, suggesting that sociality likely increased the probability of hosts encountering vectors thereby promoting parasite transmission. Foraging strata was another significant host ecological factor that increased the probability of hosts exposure to parasites (e.g. *Plasmodium*), with host species at high strata having lower *Plasmodium* prevalence compared to low strata birds. Other host traits that influenced variation in avian haemosporidian risk were associated with differences in host susceptibility to infection (e.g. sexual dimorphism and individual body condition). Interestingly, birds with better body condition had higher *Haemoproteus* prevalence than birds with poorer body condition, suggesting that birds were likely tolerant to *Haemoproteus* infection and did not suffer detrimental fitness consequences. However, further research using parasitemia data and better measures of host fitness is needed to confirm the role of tolerance in influencing disease dynamics and is another exciting research question. Finally, this study (Chapter 4) revealed that host phylogeny was an important predictor of infection risk across the Shola Sky

Island bird communities, emphasizing that future studies should account for host phylogeny when predicting variation infection risk among bird communities.

Overall, by employing a phylogenetically controlled approach, this part of my dissertation research (Chapter 4) presents a novel perspective into drivers of avian haemosporidian infection risk. First, relative importance of host ecological traits in influencing variation in infection risk across host species varies likely because *Plasmodium* and *Haemoproteus* are differentially affected by host eco-evolutionary factors. Second, my findings emphasize that including host phylogenetic relatedness in studies of association between ecological traits and infection risk is critical to gain a better understanding of drivers of variation in avian haemosporidian infection risk and for maintaining health of wildlife populations.

Prior to this research, few studies have attempted to develop genomic tools (genomics and transcriptomics approaches) to obtain high-quality parasite genomic data. However, obtaining large-scale genomic data including all three genera of avian haemosporidians and other mammalian malaria parasites has not yet been possible. Consequently, phylogenetic relationships among apicomplexan haemosporidian parasites, in general, and the evolutionary origins of avian haemosporidians, in particular, remains unresolved. Sequence capture approach based on UCE probes and high-throughput sequencing offers a promising tool to obtain parasite genomic data and build robust haemosporidian phylogenies (Chapter 5). Although, a preliminary investigation, I successfully obtained genomic data across >100 UCE loci (up to 232 loci) from clinical mammalian malaria samples and wild bird samples. This was a preliminary study but I believe, this approach could potentially allow us to obtain genomic data from other malaria parasites such as bat and ungulate malaria parasites that still lack genomic data and opens prospects for exciting

new research directions. Obtaining genomic data from a diverse group of haemosporidians will aid in comparative genomics, better understanding of host-parasite interactions, and testing phylogeographic hypotheses, such as the effects of biogeographic gaps for avian haemosporidian parasites in the Western Ghats Sky Island system.

Taken together, my dissertation research informs on the diversity, community structure, infection dynamics and genomics of avian haemosporidian parasites. This research substantially advances our understanding of avian haemosporidian ecology and evolution across a broad biogeographic scale. Tropical avian haemosporidians, particularly outside of the Western Hemisphere have been poorly studied relative to their temperate counterparts and although my work herein filled in some of the knowledge gaps, many questions remain unanswered. Future studies are needed to decipher the bird-parasite-vector tripartite interaction from the vector's perspective and in the context of anthropogenic influences such as habitat fragmentation and global climate change. We still lack information on the fitness effects of avian haemosporidians in the Western Ghats avian communities which presents an important area of investigation for future research. This will be critical from wildlife health perspective as many bird species in the Western Ghats landscape are endemic and threatened due to increased anthropogenic modifications and global climate change. Disease pressures may further exacerbate the threat and may pose severe consequences for these bird populations as parasites can exert strong selective pressure on host abundance and distribution. Consequently, further understanding fitness effects of these parasites in this ecologically important yet vulnerable tropical ecosystem may be an important step towards its conservation. In conclusion, my dissertation research provided a unique perspective on the ecological and evolutionary dynamics of vector-borne diseases in the

Western Ghats Sky Island bird community and I hope, this work will spark future research into parasite biogeography, vector biology, wildlife health, comparative genomics and biodiversity conservation.

REFERENCES

Acevedo-Whitehouse, K., Cunningham, A.A., 2006. Is MHC enough for understanding wildlife immunogenetics? Trends Ecol. Evol. 21, 433–438. https://doi.org/10.1016/j.tree.2006.05.010

Adelman, J.S., Kirkpatrick, L., Grodio, J.L., Hawley, D.M., 2013. House Finch Populations Differ in Early Inflammatory Signaling and Pathogen Tolerance at the Peak of *Mycoplasma gallisepticum* Infection. Am. Nat. 181, 674–689. https://doi.org/10.1086/670024

Agbali, M., Reichard, M., Bryjová, A., Bryja, J., Smith, C., 2010. Mate choice for nonadditive genetic benefits correlate with mhc dissimilarity in the rose bitterling (*Rhodeus ocellatus*). Evolution 64, 1683–1696. https://doi.org/10.1111/j.1558-5646.2010.00961.x

Alcala, N., Jenkins, T., Christe, P., Vuilleumier, S., 2017. Host shift and cospeciation rate estimation from co-phylogenies. Ecol. Lett. 20, 1014–1024. https://doi.org/10.1111/ele.12799

Alley, M.R., Hale, K. a, Cash, W., Ha, H.J., Howe, L., 2010. Concurrent avian malaria and avipox virus infection in translocated South Island saddlebacks (*Philesturnus carunculatus carunculatus*). N. Z. Vet. J. 58, 218–223. https://doi.org/10.1080/00480169.2010.68868

Altizer, S., Nunn, C.L., Thrall, P.H., Gittleman, J.L., Antonovics, J., Cunningham, A.A., Dobson, A.P., Ezenwa, V., Jones, K.E., Pedersen, A.B., Poss, M., Pulliam, J.R.C., 2003. Social Organization and Parasite Risk in Mammals: Integrating Theory and Empirical Studies. Annu. Rev. Ecol. Evol. Syst. 34, 517–547.

https://doi.org/10.1146/annurev.ecolsys.34.030102.151725

Altizer, S., Ostfeld, R.S., Johnson, P.T.J., Kutz, S., Harvell, C.D., 2013. Climate Change and Infectious Diseases: From Evidence to a Predictive Framework. Science 341, 514–519. https://doi.org/10.1126/science.1239401

Amundsen, P.A., Lafferty, K.D., Knudsen, R., Primicerio, R., Klemetsen, A., Kuris, A.M., 2009. Food web topology and parasites in the pelagic zone of a subarctic lake. J. Anim. Ecol. 78, 563–572. https://doi.org/10.1111/j.1365-2656.2008.01518.x

Andrews, S., 2015. FASTQC A Quality Control tool for High Throughput Sequence Data. Babraham Inst.

Arai, M., Billker, O., Morris, H.R., Panico, M., Delcroix, M., Dixon, D., Ley, S. V., Sinden, R.E., 2001. Both mosquito-derived xanthurenic acid and a host blood-derived factor regulate gametogenesis of Plasmodium in the midgut of the mosquito. Mol. Biochem. Parasitol. 116, 17–24. https://doi.org/10.1016/S0166-6851(01)00299-7

Archie, E.A., Luikart, G., Ezenwa, V.O., 2009. Infecting epidemiology with genetics: a new frontier in disease ecology. Trends Ecol. Evol. 24, 21–30. https://doi.org/10.1016/j.tree.2008.08.008

Argilla, L.S., Howe, L., D, G.B., Alley, M.R., 2013. High prevalence of Leucocytozoon spp. in the endangered Yellow-eyed Penguin (*Megadyptes antipodes*) in the sub-Antarctic regions of New Zealand. Parasitology 140, 672–682. https://doi.org/doi:10.1017/S0031182012002089

Arriero, E., Moller, A.P., 2008. Host ecology and life-history traits associated with blood parasite species richness in birds. J. Evol. Biol. 21, 1504–1513.

https://doi.org/10.1111/j.1420-9101.2008.01613.x

Asghar, M., Dennis, H., Bengt, H., Pavel, Z., Helena, W., Staffan, B., 2015. Hidden costs of infection: Chronic malaria accelerates telomere degradation and senescence in wild birds. Science 347, 9–12.

Asghar, M., Hasselquist, D., Bensch, S., 2011. Are chronic avian haemosporidian infections costly in wild birds? J. Avian Biol. 42, 530–537. https://doi.org/10.1111/j.1600-048X.2011.05281.x

Astudillo, V.G., Hernández, S.M., Kistler, W.M., Boone, S.L., Lipp, E.K., Shrestha, S., Yabsley, M.J., 2013. Spatial, temporal, molecular, and intraspecific differences of haemoparasite infection and relevant selected physiological parameters of wild birds in Georgia, USA. Int. J. Parasitol. Parasites Wildl. 2, 178–189. https://doi.org/10.1016/j.ijppaw.2013.04.005

Atkinson, C.T., Dusek, R.J., Woods, K.L., Iko, W.M., 2000. Pathogenicity of Avian Malaria in Experimentally- Infected Hawaii Amakihi. J. Wildl. Dis. 36, 197–204. https://doi.org/10.7589/0090-3558-36.2.197

Atkinson, C.T., Saili, K.S., Utzurrum, R.B., Jarvi, S.I., 2013. Experimental evidence for evolved tolerance to avian malaria in a wild population of low elevation Hawai'i 'Amakihi (Hemignathus virens). Ecohealth 10, 366–75. https://doi.org/10.1007/s10393-013-0899-2

Atkinson, C.T., Samuel, M.D., 2010. Avian malaria Plasmodium relictum in native Hawaiian forest birds: epizootiology and demographic impacts on àapapane Himatione sanguinea. J. Avian Biol. 41, 357–366. https://doi.org/10.1111/j.1600-048X.2009.04915.x

Avramenko, R.W., Redman, E.M., Lewis, R., Yazwinski, T.A., Wasmuth, J.D., Gilleard, J.S., 2015. Exploring the gastrointestinal "nemabiome": Deep amplicon sequencing to quantify

the species composition of parasitic nematode communities. PLoS One 10, 1–18. https://doi.org/10.1371/journal.pone.0143559

Ayres, J.S., Schneider, D.S., 2012. Tolerance of infections. Annu. Rev. Immunol. 30, 271–94. https://doi.org/10.1146/annurev-immunol-020711-075030

Ayres, J.S., Schneider, D.S., 2009. The Role of Anorexia in Resistance and Tolerance to Infections in Drosophila. PLoS Biol. 7, 1–10. https://doi.org/10.1371/journal.pbio.1000150

Babayan, S.A., Orton, R.J., Streicker, D.G., 2018. Predicting reservoir hosts and arthropod vectors from evolutionary signatures in RNA virus genomes. Science 362, 577–580. https://doi.org/10.1126/science.aap9072

Baele, G., Suchard, M.A., Rambaut, A., Lemey, P., 2017. Emerging concepts of data integration in pathogen phylodynamics. Syst. Biol. 66, e47–e65. https://doi.org/10.1093/sysbio/syw054

Baker, K.S., Leggett, R.M., Bexfield, N.H., Alston, M., Daly, G., Todd, S., Tachedjian, M., Holmes, C.E.G., Crameri, S., Wang, L.F., Heeney, J.L., Suu-Ire, R., Kellam, P., Cunningham, A.A., Wood, J.L.N., Caccamo, M., Murcia, P.R., 2013. Metagenomic study of the viruses of African straw-coloured fruit bats: Detection of a chiropteran poxvirus and isolation of a novel adenovirus. Virology 441, 95–106. https://doi.org/10.1016/j.virol.2013.03.014

Balbuena, J.A., Míguez-Lozano, R., Blasco-Costa, I., Naisbit, R., Alvarez, N., 2013. PACo: A Novel Procrustes Application to Cophylogenetic Analysis. PLoS One 8, e61048. https://doi.org/10.1371/journal.pone.0061048

Ballingall, K.T., McIntyre, A., Lin, Z., Timmerman, N., Matthysen, E., Lurz, P.W.W., Melville, L., Wallace, A., Meredith, A.L., Romeo, C., Wauters, L.A., Sainsbury, A.W., McInnes,

C.J., 2016. Limited diversity associated with duplicated class II MHC-DRB genes in the red squirrel population in the United Kingdom compared with continental Europe. Conserv. Genet. 17, 1171–1182. https://doi.org/10.1007/s10592-016-0852-3

Barrow, L.N., Allen, J.M., Huang, X., Bensch, S., Witt, C.C., 2018. Genomic sequence capture of haemosporidian parasites: Methods and prospects for enhanced study of host-parasite evolution. Mol. Ecol. Resour. 19, 400–410. https://doi.org/10.1111/1755-0998.12977

Barrow, L.N., McNew, S.M., Mitchell, N., Galen, S.C., Lutz, H.L., Skeen, H., Valqui, T., Weckstein, J.D., Witt, C.C., 2019. Deeply conserved susceptibility in a multi-host, multi-parasite system. Ecol. Lett. 22, 987–998. https://doi.org/10.1111/ele.13263

Bass, D., Stentiford, G.D., Littlewood, D.T.J., Hartikainen, H., 2015. Diverse Applications of Environmental DNA Methods in Parasitology. Trends Parasitol. 31, 499–513. https://doi.org/10.1016/j.pt.2015.06.013

Bateson, Z.W., Hammerly, S.C., Johnson, J.A., Morrow, M.E., Whittingham, L.A., Dunn, P.O., 2016. Specific alleles at immune genes, rather than genome-wide heterozygosity, are related to immunity and survival in the critically endangered Attwater's prairie-chicken. Mol. Ecol. 25, 4730–4744. https://doi.org/10.1111/mec.13793

Beadell, J.S., Covas, R., Gebhard, C., Ishtiaq, F., Melo, M., Schmidt, B.K., Perkins, S.L., Graves, G.R., Fleischer, R.C., 2009. Host associations and evolutionary relationships of avian blood parasites from West Africa. Int. J. Parasitol. 39, 257–266. https://doi.org/10.1016/j.ijpara.2008.06.005

Beasley, D.A.E., Bonisoli-Alquati, A., Mousseau, T.A., 2013. The use of fluctuating asymmetry as a measure of environmentally induced developmental instability: A meta-analysis. Ecol.

Indic. 30, 218–226. https://doi.org/10.1016/j.ecolind.2013.02.024

Becker, M.H., Walke, J.B., Cikanek, S., Savage, A.E., Mattheus, N., Santiago, C.N., Minbiole, K.P.C., Harris, R.N., Belden, L.K., Gratwicke, B., 2015. Composition of symbiotic bacteria predicts survival in Panamanian golden frogs infected with a lethal fungus. Proc. R. Soc. B Biol. Sci. 282. https://doi.org/10.1098/rspb.2014.2881

Beldomenico, P.M., Begon, M., 2010. Disease spread, susceptibility and infection intensity: vicious circles? Trends Ecol. Evol. 25, 21–27. https://doi.org/10.1016/j.tree.2009.06.015

Bensch, S., Canback, B., DeBarry, J.D., Johansson, T., Hellgren, O., Kissinger, J.C., Palinauskas, V., Videvall, E., Valkiunas, G., 2016a. The Genome of Haemoproteus tartakovskyi and Its Relationship to Human Malaria Parasites. Genome Biol. Evol. 8, 1361–1373. https://doi.org/10.1093/gbe/evw081

Bensch, S., Canbäck, B., DeBarry, J.D., Johansson, T., Hellgren, O., Kissinger, J.C., Palinauskas, V., Videvall, E., Valkiūnas, G., 2016b. The Genome of *Haemoproteus tartakovskyi* and Its Relationship to Human Malaria Parasites. Genome Biol. Evol. 8, 1361–1373. https://doi.org/10.1093/gbe/evw081

Bensch, S., Hellgren, O., Perez-Tris, J., 2009. MalAvi: a public database of malaria parasites and related haemosporidians in avian hosts based on mitochondrial cytochrome b lineages. Mol. Ecol. Resour. 9, 1353–8. https://doi.org/10.1111/j.1755-0998.2009.02692.x

Bensch, S., Perez-Tris J, Waldstrom, J., Hellgren, O., 2004. Linkage Between Nuclear and Mitochondrial Dna Sequences in Avian Malaria Parasites : Multiple Cases of Cryptic Speciation ? Evolution 58, 1617–1621. https://doi.org/10.1111/j.0014-3820.2004.tb01742.x

Berenger, J.-M., Parola, P., 2016. Arthropod Vectors of Medical Importance, in: Infectious

Diseases. Elsevier, pp. 104–112. https://doi.org/10.1016/b978-0-7020-6285-8.00012-5

Bergner, L.M., Orton, R.J., da Silva Filipe, A., Shaw, A.E., Becker, D.J., Tello, C., Biek, R., Streicker, D.G., 2019. Using noninvasive metagenomics to characterize viral communities from wildlife. Mol. Ecol. Resour. 19, 128–143. https://doi.org/10.1111/1755-0998.12946

Bermejo, M., Rodríguez-Teijeiro, J.D., Illera, G., Barroso, A., Vilà, C., Walsh, P.D., 2006. Ebola outbreak killed 5000 gorillas. Science 314, 1564. https://doi.org/10.1126/science.1133105

Biek, R., Drummond, A.J., Poss, M., 2006. A Virus Reveals Population Structure and Recent Demographic History of Its Carnivore Host. Science 311, 538–541. https://doi.org/10.1126/science.1121360

Biek, R., O'hare, A., Wright, D., Mallon, T., Mccormick, C., 2012. Whole Genome Sequencing Reveals Local Transmission Patterns of Mycobacterium bovis in Sympatric Cattle and Badger Populations. PLoS Pathog. 8, 1003008. https://doi.org/10.1371/journal.ppat.1003008

Biek, R., Real, L.A., 2010. The landscape genetics of infectious disease emergence and spread. Mol. Ecol. 19, 3515–3531. https://doi.org/10.1111/j.1365-294X.2010.04679.x

Bird, B.H., Mazet, J.A.K., 2018. Detection of Emerging Zoonotic Pathogens: An Integrated One Health Approach. Annu. Rev. Anim. Biosci. 6, 121–139. https://doi.org/10.1146/annurev-animal-030117-014628

Blackburn, T.M., Ewen, J.G., 2017. Parasites as Drivers and Passengers of Human-Mediated Biological Invasions. Ecohealth 14, 61–73. https://doi.org/10.1007/s10393-015-1092-6

Blakeslee, A.M.H., Byers, J.E., 2008. Using parasites to inform ecological history: Comparisons among three congeneric marine snails. Ecology 89, 1068–1078. https://doi.org/10.1890/07-

0832.1

Blanar, C.A., Munkittrick, K.R., Houlahan, J., MacLatchy, D.L., Marcogliese, D.J., 2009. Pollution and parasitism in aquatic animals: A meta-analysis of effect size. Aquat. Toxicol. 93, 18–28. https://doi.org/10.1016/j.aquatox.2009.03.002

Blanchong, J.A., Scribner, K.T., Kravchenko, A.N., Winterstein, S.R., 2007. TB-infected deer are more closely related than non-infected deer. Biol. Lett. 3, 104–106. https://doi.org/10.1098/rsbl.2006.0547

Blaustein, A.R., Gervasi, S.S., Johnson, P.T.J., Hoverman, J.T., Belden, L.K., Bradley, P.W., Xie, G.Y., 2012. Ecophysiology meets conservation: Understanding the role of disease in amphibian population declines. Philos. Trans. R. Soc. B Biol. Sci. 367, 1688–1707. https://doi.org/10.1098/rstb.2012.0011

Blehert, D.S., Hicks, A.C., Behr, M., Meteyer, C.U., Berlowski-Zier, B.M., Buckles, E.L., Coleman, J.T.H., Darling, S.R., Gargas, A., Niver, R., Okoniewski, J.C., Rudd, R.J., Stone, W.B., 2009. Bat white-nose syndrome: An emerging fungal pathogen? Science 323, 227. https://doi.org/10.1126/science.1163874

Bletz, M.C., Loudon, A.H., Becker, M.H., Bell, S.C., Woodhams, D.C., Minbiole, K.P.C., Harris, R.N., 2013. Mitigating amphibian chytridiomycosis with bioaugmentation: characteristics of effective probiotics and strategies for their selection and use. Ecol. Lett. 16, 807–820. https://doi.org/10.1111/ele.12099

Blüthgen, N., Fründ, J., Vazquez, D.P., Menzel, F., 2008. What do interaction network metrics tell us about specialization and biological traits? Ecology. https://doi.org/10.1890/07-2121.1

Bodewes, R., Ruiz-Gonzalez, A., Schapendonk, C.M.E., Van Den Brand, J.M.A., Osterhaus,

A.D.M.E., Smits, S.L., 2014. Viral metagenomic analysis of feces of wild small carnivores. Virol. J. 11, 1–13. https://doi.org/10.1186/1743-422X-11-89

Bohmann, K., Evans, A., Gilbert, M.T.P., Carvalho, G.R., Creer, S., Knapp, M., Yu, D.W., de Bruyn, M., 2014. Environmental DNA for wildlife biology and biodiversity monitoring. Trends Ecol. Evol. https://doi.org/10.1016/j.tree.2014.04.003

Bohmann, K., Schnell, I.B., Gilbert, M.T.P., 2013. When bugs reveal biodiversity. Mol. Ecol. 22, 909–911. https://doi.org/10.1111/mec.12221

Böhme, U., Otto, T.D., Cotton, J.A., Steinbiss, S., Sanders, M., Oyola, S.O., Nicot, A., Gandon, S., Patra, K.P., Herd, C., Bushell, E., Modrzynska, K.K., Billker, O., Vinetz, J.M., Rivero, A., Newbold, C.I., Berriman, M., 2018. Complete avian malaria parasite genomes reveal features associated with lineage-specific evolution in birds and mammals. Genome Res. 28, 547–560. https://doi.org/10.1101/gr.218123.116

Bolger, A.M., Lohse, M., Usadel, B., 2014. Trimmomatic: a flexible trimmer for Illumina sequence data 30, 2114–2120. https://doi.org/10.1093/bioinformatics/btu170

Bolker, B., Claude, J., Sien, H., Des-, R., Durand, B., Dutheil, J., Gas-, O., Heibl, C., Lawson, D., Lefort, V., Legen-, P., Lemon, J., Nylander, J., Opgen-rhein, R., 2014. Package “ape.” Anal. Phylogenetics Evol. https://doi.org/10.1093/bioinformatics/btg412

Boots, M., Sasaki, A., 2003. Parasite evolution and extinctions. Ecol. Lett. 6, 176–182. https://doi.org/10.1046/j.1461-0248.2003.00426.x

Borner, J., Pick, C., Thiede, J., Kolawole, O.M., Kingsley, M.T., Schulze, J., Cottontail, V.M., Wellinghausen, N., Schmidt-Chanasit, J., Bruchhaus, I., Burmester, T., 2015. Phylogeny of haemosporidian blood parasites revealed by a multi-gene approach. Mol. Phylogenet. Evol.

94, 221–231. https://doi.org/10.1016/j.ympev.2015.09.003

Bouyer, J., Dicko, A.H., Cecchi, G., Ravel, S., Guerrini, L., Solano, P., Vreysen, M.J.B., De Meeûs, T., Lancelot, R., 2015. Mapping landscape friction to locate isolated tsetse populations that are candidates for elimination. Proc. Natl. Acad. Sci. 112, 14575–14580. https://doi.org/10.1073/pnas.1516778112

Brambilla, A., Keller, L., Bassano, B., Grossen, C., 2018. Heterozygosity–fitness correlation at the major histocompatibility complex despite low variation in Alpine ibex (*Capra ibex*). Evol. Appl. 11, 631–644. https://doi.org/10.1111/eva.12575

Brandt, A.L., Green, M.L., Ishida, Y., Roca, A.L., Novakofski, J., Mateus-Pinilla, N.E., 2018. Influence of the geographic distribution of prion protein gene sequence variation on patterns of chronic wasting disease spread in white-tailed deer (*Odocoileus virginianus*). Prion 12, 204–215. https://doi.org/10.1080/19336896.2018.1474671

Briggs, J.C., Collections, Z., Museum, G., 2003. The biogeographic and tectonic history of India 381–388.

Brook, R.K., Wal, E. Vander, van Beest, F.M., McLachlan, S.M., 2012. Evaluating use of cattle winter feeding areas by elk and white-tailed deer: Implications for managing bovine tuberculosis transmission risk from the ground up. Prev. Vet. Med. 108, 137–147. https://doi.org/10.1016/j.prevetmed.2012.07.017

Brooks, D.R., Hoberg, E.P., 2013. The emerging infectious diseases crisis and pathogen pollution. Balanc. Nat. Hum. impact 215–229. https://doi.org/10.1017/CBO9781139095075.022

Brown, B.L., Creed, R.P., Skelton, J., Rollins, M.A., Farrell, K.J., 2012. The fine line between

mutualism and parasitism: Complex effects in a cleaning symbiosis demonstrated by multiple field experiments. Oecologia 170, 199–207. https://doi.org/10.1007/s00442-012-2280-5

Brunker, K., Lemey, P., Marston, D.A., Fooks, A.R., Lugelo, A., Ngeleja, C., Hampson, K., Biek, R., 2018. Landscape attributes governing local transmission of an endemic zoonosis: Rabies virus in domestic dogs. Mol. Ecol. 27, 773–788. https://doi.org/10.1111/mec.14470

Buck, J.C., Ripple, W.J., 2017. Infectious Agents Trigger Trophic Cascades. Trends Ecol. Evol. 32, 681–694. https://doi.org/10.1016/j.tree.2017.06.009

Budischak, S.A., O'Neal, D., Jolles, A.E., Ezenwa, V.O., 2018. Differential host responses to parasitism shape divergent fitness costs of infection. Funct. Ecol. 32, 324–333. https://doi.org/10.1111/1365-2435.12951

Bull, C.M., Godfrey, S.S., Gordon, D.M., 2012. Social networks and the spread of Salmonella in a sleepy lizard population. Mol. Ecol. 21, 4386–4392. https://doi.org/10.1111/j.1365-294X.2012.05653.x

Cable, J., Barber, I., Boag, B., Ellison, A.R., Morgan, E.R., Murray, K., Pascoe, E.L., Sait, S.M., Wilson, A.J., Booth, M., 2017. Global change, parasite transmission and disease control: Lessons from ecology. Philos. Trans. R. Soc. B Biol. Sci. 372. https://doi.org/10.1098/rstb.2016.0088

Caillaud, D., Craft, M.E., Meyers, L.A., 2013. Epidemiological effects of group size variation in social species. J. R. Soc. Interface 10. https://doi.org/10.1098/rsif.2013.0206

Caillaud, D., Levréro, F., Cristescu, R., Gatti, S., Dewas, M., Douadi, M., Gautier-Hion, A., Raymond, M., Ménard, N., 2006. Gorilla susceptibility to Ebola virus: The cost of sociality.

Curr. Biol. 16, 489–491. https://doi.org/10.1103/PhysRevLett.107.261801

Caldwell, A., Siddle, H. V., 2017. The role of MHC genes in contagious cancer: the story of Tasmanian devils. Immunogenetics 69, 537–545. https://doi.org/10.1007/s00251-017-0991-9

Caldwell, R.M., Schafer, J.F., Compton, L.E., Patterson, F.L., 1958. Tolerance to Cereal Leaf Rusts. Science 128, 714–715.

Calegaro-Marques, C., Amato, S.B., 2014. Urbanization breaks up host-parasite interactions: A case study on parasite community ecology of rufous-bellied thrushes (*Turdus rufiventris*) along a rural-urban gradient. PLoS One 9. https://doi.org/10.1371/journal.pone.0103144

Campbell, F., Strang, C., Ferguson, N., Cori, A., Jombart, T., 2018. When are pathogen genome sequences informative of transmission events? PLoS Pathog. 14. https://doi.org/10.1371/journal.ppat.1006885

Carlton, J.M., Adams, J.H., Silva, J.C., Bidwell, S.L., Lorenzi, H., Caler, E., Crabtree, J., Angiuoli, S. V., Merino, E.F., Amedeo, P., Cheng, Q., Coulson, R.M.R., Crabb, B.S., Del Portillo, H.A., Essien, K., Feldblyum, T. V., Fernandez-Becerra, C., Gilson, P.R., Gueye, A.H., Guo, X., Kang'A, S., Kooij, T.W.A., Korsinczky, M., Meyer, E.V.S., Nene, V., Paulsen, I., White, O., Ralph, S.A., Ren, Q., Sargeant, T.J., Salzberg, S.L., Stoeckert, C.J., Sullivan, S.A., Yamamoto, M.M., Hoffman, S.L., Wortman, J.R., Gardner, M.J., Galinski, M.R., Barnwell, J.W., Fraser-Liggett, C.M., 2008. Comparative genomics of the neglected human malaria parasite Plasmodium vivax. Nature 455, 757–763. https://doi.org/10.1038/nature07327

Carlton, J.M., Angiuoli, S. V., Suh, B.B., Kooij, T.W., Pertea, M., Silva, J.C., Ermolaeva, M.D.,

Allen, J.E., Selengut, J.D., Koo, H.L., Peterson, J.D., Pop, M., Kosack, D.S., Shumway, M.F., Bidwell, S.L., Shallom, S.J., Van Aken, S.E., Riedmuller, S.B., Feldblyum, T. V., Cho, J.K., Quackenbush, J., Sedegah, M., Shoaibi, A., Cummings, L.M., Florens, L., Yates, J.R., Raine, J.D., Sinden, R.E., Harris, M.A., Cunningham, D.A., Preiser, P.R., Bergman, L.W., Vaidya, A.B., Van Lin, L.H., Janse, C.J., Waters, A.P., Smith, H.O., White, O.R., Salzberg, S.L., Venter, J.C., Fraser, C.M., Hoffman, S.L., Gardner, M.J., Carucci, D.J., 2002. Genome sequence and comparative analysis of the model rodent malaria parasite Plasmodium yoelii yoelii. Nature 419, 512–519. https://doi.org/10.1038/nature01099

Carlton, J.M., Perkins, S.L., Deitsch, K.W., 2013. Malaria Parasites: Comparative genomics, evolution and molecular biology. Caister Academic Press.

Carpi, G., Cagnacci, F., Wittekindt, N.E., Zhao, F., Qi, J., Tomsho, L.P., Drautz, D.I., Rizzoli, A., Schuster, S.C., 2011. Metagenomic profile of the bacterial communities associated with Ixodes ricinus ticks. PLoS One 6. https://doi.org/10.1371/journal.pone.0025604

Cassin-Sackett, L., Callicrate, T.E., Fleischer, R.C., 2019. Parallel evolution of gene classes, but not genes: Evidence from Hawai'ian honeycreeper populations exposed to avian malaria. Mol. Ecol. 28, 568–583. https://doi.org/10.1111/mec.14891

Castresana, J., 2000. Selection of conserved blocks from multiple alignments for their use in phylogenetic analysis. Mol. Biol. Evol. 17, 540–552. https://doi.org/10.1093/oxfordjournals.molbev.a026334

CEPF, 2007. Western Ghats & Sri Lanka biodiversity hotspot, Critical Ecosystem Partnership fund.

Cerny, O., Votypka, J., Svobodova, M., 2011. Spatial feeding preferences of ornithophilic

mosquitoes, blackflies and biting midges. Med. Vet. Entomol. 25, 104–108. https://doi.org/10.1111/j.1365-2915.2010.00875.x

Charleston, M. a, Robertson, D.L., 2002. Preferential host switching by primate lentiviruses can account for phylogenetic similarity with the primate phylogeny. Syst. Biol. 51, 528–535. https://doi.org/10.1080/10635150290069940

Charleston, M.A., Robertson, D.L., Sanderson, M., 2002. Preferential Host Switching by Primate Lentiviruses Can Account for Phylogenetic Similarity with the Primate Phylogeny. Syst. Biol. 51, 528–535. https://doi.org/10.1080/10635150290069940

Chasar, A., Loiseau, C., Valkiunas, G., Iezhova, T., Smith, T.B., Sehgal, R.N.M., 2009. Prevalence and diversity patterns of avian blood parasites in degraded African rainforest habitats. Mol. Ecol. 18, 4121–4133. https://doi.org/10.1111/j.1365-294X.2009.04346.x

Chatterjee, S., Goswami, A., Scotese, C.R., 2013. The longest voyage : Tectonic , magmatic , and paleoclimatic evolution of the Indian plate during its northward fl ight from Gondwana to Asia. Gondwana Res. 23, 238–267. https://doi.org/10.1016/j.gr.2012.07.001

Chen, I., Hill, J.K., Ohlemüller, R., Roy, D.B., Thomas, C.D., 2011. Rapid range shifts of species Associated with High Levels of climate warming. Science 333, 1024–1026. https://doi.org/10.1126/science.1206432

Chen, W., Bei, Y., Li, H., 2015. Genetic variation of the major histocompatibility complex (MHC class II B gene) in the threatened Hume's pheasant, Syrmaticus humiae. PLoS One 10, 1–12. https://doi.org/10.1371/journal.pone.0116499

Cheng, Y.C., Musiani, M., Cavedon, M., Gilch, S., 2017. High prevalence of prion protein genotype associated with resistance to chronic wasting disease in one Alberta woodland

caribou population. Prion 11, 136–142. https://doi.org/10.1080/19336896.2017.1300741

Chernin, E., 1968. Interference with the Capacity of Schistosoma mansoni Miracidia to Infect the Molluscan Host. J. Parasitol. 54, 509. https://doi.org/10.2307/3277073

Chiu, L., Bazin, T., Truchetet, M.E., Schaeverbeke, T., Delhaes, L., Pradeu, T., 2017. Protective microbiota: From localized to long-reaching co-immunity. Front. Immunol. https://doi.org/10.3389/fimmu.2017.01678

Chiyo, P.I., Grieneisen, L.E., Wittemyer, G., Moss, C.J., Lee, P.C., Douglas-Hamilton, I., Archie, E.A., 2014. The influence of social structure, habitat, and host traits on the transmission of Escherichia coli in wild elephants. PLoS One 9. https://doi.org/10.1371/journal.pone.0093408

Christou, L., 2011. The global burden of bacterial and viral zoonotic infections. Clin. Microbiol. Infect. https://doi.org/10.1111/j.1469-0691.2010.03441.x

Cincotta, R.P., Wisnewski, J., Engelman, R., 2000. Human population in the biodiversity hotspots. Nature 404, 990–992. https://doi.org/10.1038/35010105

Civitello, D.J., Cohen, J., Fatima, H., Halstead, N.T., Liriano, J., McMahon, T.A., Ortega, C.N., Sauer, E.L., Sehgal, T., Young, S., Rohr, J.R., 2015. Biodiversity inhibits parasites: Broad evidence for the dilution effect. Proc. Natl. Acad. Sci. 112, 8667–8671. https://doi.org/10.1073/pnas.1506279112

Clark, N.J., 2018. Phylogenetic uniqueness, not latitude, explains the diversity of avian blood parasite communities worldwide. Glob. Ecol. Biogeogr. 744–755. https://doi.org/10.1111/geb.12741

Clark, N.J., Clegg, S.M., Lima, M.R., 2014. A review of global diversity in avian

haemosporidians (Plasmodium and Haemoproteus: Haemosporida): new insights from molecular data. Int. J. Parasitol. 44, 329–38. https://doi.org/10.1016/j.ijpara.2014.01.004

Cleaveland, S., Packer, C., Hampson, K., Kaare, M., Kock, R., Craft, M., Limbo, T., Mlengeya, T., Dobson, A., 2009. The Multiple Roles of Infectious Diseases in the Serengeti Ecosystem, in: Sinclair, A.R.E., Packer, C., Mduma, S.A.R., Fryxell, J.M. (Eds.), Serengeti III: Human Impacts on Ecosystem Dynamics. pp. 209–241. https://doi.org/10.7208/chicago/9780226760353.003.0007

Coates, A., Barnett, L.K., Hoskin, C., Phillips, B.L., 2017. Living on the Edge: Parasite Prevalence Changes Dramatically across a Range Edge in an Invasive Gecko. Am. Nat. 189, 178–183. https://doi.org/10.1086/689820

Cobb, R.C., Chan, M.N., Meentemeyer, R.K., Rizzo, D.M., 2012. Common Factors Drive Disease and Coarse Woody Debris Dynamics in Forests Impacted by Sudden Oak Death. Ecosystems 15, 242–255. https://doi.org/10.1007/s10021-011-9506-y

Colwell, R.K., Brehm, G., Cardelús, C.L., Gilman, A.C., Longino, J.T., 2008. Global Warming, Elevational Range Shifts, and Lowland Biotic Attrition in the Wet Tropics. Science 322, 258–261. https://doi.org/10.1126/science.1162547

Connelly, S., Pringle, C.M., Bixby, R.J., Brenes, R., Whiles, M.R., Lips, K.R., Kilham, S., Huryn, A.D., 2008. Changes in stream primary producer communities resulting from large-scale catastrophic amphibian declines: Can small-scale experiments predict effects of tadpole loss? Ecosystems 11, 1262–1276. https://doi.org/10.1007/s10021-008-9191-7

Conow, C., Fielder, D., Ovadia, Y., Libeskind-Hadas, R., 2010. Jane: a new tool for the cophylogeny reconstruction problem. Algorithms Mol. Biol. 5, 16.

https://doi.org/10.1186/1748-7188-5-16

Côté, S.D., Stien, A., Irvine, R.J., Dallas, J.F., Marshall, F., Halvorsen, O., Langvatn, R., Albon, S.D., 2005. Resistance to abomasal nematodes and individual genetic variability in reindeer. Mol. Ecol. 14, 4159–4168. https://doi.org/10.1111/j.1365-294X.2005.02733.x

Crabtree, M.B., Kading, R.C., Mutebi, J.-P., Lutwama, J.J., Miller, B.R., 2013. Identification of Host Blood From Engorged Mosquitoes Collected in Western Uganda Using Cytochrome Oxidase I Gene Sequences. J. Wildl. Dis. 49, 611–626. https://doi.org/10.7589/2012-08-213

Craft, M.E., 2015. Infectious disease transmission and contact networks in wildlife and livestock. Philos. Trans. R. Soc. B Biol. Sci. 370. https://doi.org/10.1063/1.4933351

Crispell, J., Benton, C.H., Balaz, D., De Maio, N., Ahkmetova, A., Allen, A., Biek, R., Presho, E.L., Dale, J., Hewinson, G., Lycett, S.J., Nunez-Garcia, J., Skuce, R.A., Trewby, H., Wilson, D.J., Zadoks, R.N., Delahay, R.J., Kao, R.R., 2019. Combining genomics and epidemiology to analyse bi-directional transmission of Mycobacterium bovis in a multi-host system. Elife 8, 1–36. https://doi.org/10.7554/eLife.45833

Cullingham, C.I., Merrill, E.H., Pybus, M.J., Bollinger, T.K., Wilson, G.A., Coltman, D.W., 2011a. Broad and fine-scale genetic analysis of white-tailed deer populations: Estimating the relative risk of chronic wasting disease spread. Evol. Appl. 4, 116–131. https://doi.org/10.1111/j.1752-4571.2010.00142.x

Cullingham, C.I., Nakada, S.M., Merrill, E.H., Bollinger, T.K., Pybus, M.J., Coltman, D.W., 2011b. Multiscale population genetic analysis of mule deer (Odocoileus hemionus hemionus) in western Canada sheds new light on the spread of chronic wasting disease. Can. J. Zool. 89, 134–147. https://doi.org/10.1139/Z10-104

D’Cruz, E., Nair, P.K.R., Prasannakumar, V., 2000. Palghat Gap - A Dextral Shear Zone from the South Indian Granulite Terrain. Gondwana Res. 3, 21–31. https://doi.org/10.1016/s1342-937x(05)70054-x

Dadam, D., Robinson, R.A., Clements, A., Peach, W.J., Bennett, M., Rowcliffe, J.M., Cunningham, A.A., 2019. Avian malaria-mediated population decline of a widespread iconic bird species. R. Soc. Open Sci. 6, 182197. https://doi.org/10.1098/rsos.182197

Darriba, D., Taboada, G.L., Doallo, R., Posada, D., 2012. jModelTest 2: more models, new heuristics and parallel computing. Nat. Methods 9, 772–772. https://doi.org/10.1038/nmeth.2109

Das, A., Nagendra, H., Anand, M., Bunyan, M., 2015. Topographic and bioclimatic determinants of the occurrence of forest and grassland in tropical montane forest-grassland mosaics of the Western Ghats, India. PLoS One 10, 1–19. https://doi.org/10.1371/journal.pone.0130566

Daszak, P., Cunningham, A., Hyatt, A., 2000. Emerging Infectious Diseases of Wildlife - Threats to Biodiversity and Human Health. Science 287, 443–449. https://doi.org/10.1126/science.287.5452.443

De Castro, F., Bolker, B., 2005. Mechanisms of disease-induced extinction. Ecol. Lett. 8, 117–126. https://doi.org/10.1111/j.1461-0248.2004.00693.x

De Roode, J.C., Lefèvre, T., 2012. Behavioral immunity in insects. Insects 3, 789–820. https://doi.org/10.3390/insects3030789

de Villemereuil, P., Gimenez, O., Doligez, B., 2013. Comparing parent-offspring regression with frequentist and Bayesian animal models to estimate heritability in wild populations: a simulation study for Gaussian and binary traits. Methods Ecol. Evol. 4, 260–275.

https://doi.org/10.1111/2041-210X.12011

Decaestecker, E., Gaba, S., Raeymaekers, J.A.M., Stoks, R., Van Kerckhoven, L., Ebert, D., De Meester, L., 2007. Host-parasite “Red Queen” dynamics archived in pond sediment. Nature 450, 870–873. https://doi.org/10.1038/nature06291

DeCandia, A.L., Dobson, A.P., VonHoldt, B.M., 2018. Toward an integrative molecular approach to wildlife disease. Conserv. Biol. 32, 798–807. https://doi.org/10.1111/cobi.13083

Dellicour, S., Rose, R., Pybus, O.G., 2016. Explaining the geographic spread of emerging epidemics: A framework for comparing viral phylogenies and environmental landscape data. BMC Bioinformatics 17, 1–12. https://doi.org/10.1186/s12859-016-0924-x

Derne, B.T., Hutchinson, M.N., Weinstein, P., Gardner, M.G., Halliday, B., 2018. Parasite in peril? A new species of mite in the genus Ophiomegistus Banks (Parasitiformes: Paramegistidae) on an endangered host, the pygmy bluetongue lizard Tiliqua adelaidensis (Peters) (Squamata: Scincidae). Austral Ecol. 44, 420–432. https://doi.org/10.1111/aec.12692

Deyoung, R.W., Zamorano, A., Mesenbrink, B.T., Campbell, T.A., Leland, B.R., Moore, G.M., Honeycutt, R.L., Root, J.J., 2009. Landscape-Genetic Analysis of Population Structure in the Texas Gray Fox Oral Rabies Vaccination Zone. J. Wildl. Manage. 73, 1292–1299. https://doi.org/10.2193/2008-336

Dharmarajan, G., 2015. Inbreeding in stochastic subdivided mating systems: the genetic consequences of host spatial structure, aggregated transmission dynamics and life history characteristics in parasite populations. J. Genet. 94, 43–53. https://doi.org/10.1007/s12041-

015-0488-y

Dharmarajan, G., Beasley, J.C., Beatty, W.S., Olson, Z.H., Fike, J.A., Rhodes, O.E., Park, A., 2016. Genetic co-structuring in host-parasite systems: Empirical data from raccoons and raccoon ticks. Ecosphere 7, 1–15. https://doi.org/10.1002/ecs2.1269

Dharmarajan, G., Beasley, J.C., Fike, J.A., Raizman, E.A., Wu, C.C., Pogranichniy, R.M., Rhodes, O.E., 2012. Effects of kin-structure on disease dynamics in raccoons (Procyon lotor) inhabiting a fragmented landscape. Basic Appl. Ecol. 13, 560–567. https://doi.org/10.1016/j.baae.2012.08.010

Dharmarajan, G., Beasley, J.C., Rhodes, O.E., 2011. Heterozygote deficiencies in parasite populations: an evaluation of interrelated hypotheses in the raccoon tick, Ixodes texanus. Heredity 106, 253–260. https://doi.org/10.1038/hdy.2010.84

Dharmarajan, G., Beasley, J.C., Rhodes, O.E., 2010. Spatial and temporal factors affecting parasite genotypes encountered by hosts: Empirical data from American dog ticks (Dermacentor variabilis) parasitising raccoons (Procyon lotor). Int. J. Parasitol. 40, 787–795. https://doi.org/10.1016/j.ijpara.2009.12.004

Dharmarajan, G., Walker, K.D., Lehmann, T., 2019. Variation in Tolerance to Parasites Affects Vectorial Capacity of Natural Asian Tiger Mosquito Populations. Curr. Biol. 29, 3946–3952. https://doi.org/10.1016/j.cub.2019.09.047

Dheilly, N.M., Bolnick, D., Bordenstein, S., Brindley, P.J., Figuères, C., Holmes, E.C., Martínez Martínez, J., Phillips, A.J., Poulin, R., Rosario, K., 2017. Parasite Microbiome Project: Systematic Investigation of Microbiome Dynamics within and across Parasite-Host Interactions. MSystems 2, e00050-17. https://doi.org/10.1128/msystems.00050-17

Dheilly, N.M., Martínez Martínez, J., Rosario, K., Brindley, P.J., Fichorova, R.N., Kaye, J.Z., Kohl, K.D., Knoll, L.J., Lukeš, J., Perkins, S.L., Poulin, R., Schriml, L., Thompson, L.R., 2019. Parasite microbiome project: Grand challenges. PLoS Pathog. 15, e1008028. https://doi.org/10.1371/journal.ppat.1008028

Dhondt, A.A., Altizer, S., Cooch, E.G., Davis, A.K., Dobson, A., Driscoll, M.J.L., Hartup, B.K., Hawley, D.M., Hochachka, W.M., Hosseini, P.R., Jennelle, C.S., Kollias, G. V., Ley, D.H., Swarthout, E.C.H., Sydenstricker, K. V., 2005. Dynamics of a novel pathogen in an avian host: Mycoplasmal conjunctivitis in house finches. Acta Trop. 94, 77–93. https://doi.org/10.1016/j.actatropica.2005.01.009

Dirzo, R., Young, H.S., Galetti, M., Ceballos, G., Isaac, N.J.B., Collen, B., 2014. Defaunation in the Anthropocene. Science 345, 401–406. https://doi.org/10.1126/science.1251817

Dobson, A., Crawley, M., 1994. Pathogens and the structure of plant communities. Trends Ecol. Evol. 9, 393–398. https://doi.org/10.1016/0169-5347(94)90062-0

Dobson, A., Lafferty, K.D., Kuris, A.M., Hechinger, R.F., Jetz, W., 2008. Homage to Linnaeus: How many parasites? How many hosts? Proc. Natl. Acad. Sci. 105, 11482–11489. https://doi.org/10.1073/pnas.0803232105

Dorigatti, I., McCormack, C., Nedjati-Gilani, G., Ferguson, N.M., 2018. Using Wolbachia for Dengue Control: Insights from Modelling. Trends Parasitol. 34, 102–113. https://doi.org/10.1016/j.pt.2017.11.002

Drees, K.P., Lorch, J.M., Puechmaille, S.J., Parise, K.L., Wibbelt, G., Hoyt, J.R., Sun, K., Jargalsaikhan, A., Dalannast, M., Palmer, J.M., Lindner, D.L., Kilpatrick, A.M., Pearson, T., Keim, P.S., Blehert, D.S., Foster, J.T., 2017. Phylogenetics of a Fungal Invasion:

Origins and Widespread Dispersal of White-Nose Syndrome. MBio 8, e01941-17. https://doi.org/10.1128/mBio

Dudas, G., Bedford, T., 2019. The ability of single genes vs full genomes to resolve time and space in outbreak analysis. BMC Evol. Biol. 19, 1–17. https://doi.org/10.1186/s12862-019-1567-0

Dunn, A.M., Hatcher, M.J., 2015. Parasites and biological invasions: Parallels, interactions, and control. Trends Parasitol. 31, 189–199. https://doi.org/10.1016/j.pt.2014.12.003

Dunn, A.M., Torchin, M.E., Hatcher, M.J., Kotanen, P.M., Blumenthal, D.M., Byers, J.E., Coon, C.A.C., Frankel, V.M., Holt, R.D., Hufbauer, R.A., Kanarek, A.R., Schierenbeck, K.A., Wolfe, L.M., Perkins, S.E., 2012. Indirect effects of parasites in invasions. Funct. Ecol. 26, 1262–1274. https://doi.org/10.1111/j.1365-2435.2012.02041.x

Dunn, R.R., Harris, N.C., Colwell, R.K., Koh, L.P., Sodhi, N.S., 2009. The sixth mass coextinction: Are most endangered species parasites and mutualists? Proc. R. Soc. B Biol. Sci. 276, 3037–3045. https://doi.org/10.1098/rspb.2009.0413

Duvallet, C., Gibbons, S.M., Gurry, T., Irizarry, R.A., Alm, E.J., 2017. Meta-analysis of gut microbiome studies identifies disease-specific and shared responses. Nat. Commun. 8, 1–10. https://doi.org/10.1038/s41467-017-01973-8

Eastwood, J.R., Ribot, R.F.H., Rollins, L.A., Buchanan, K.L., Walder, K., Bennett, A.T.D., Berg, M.L., 2017. Host heterozygosity and genotype rarity affect viral dynamics in an avian subspecies complex. Sci. Rep. 7, 1–11. https://doi.org/10.1038/s41598-017-13476-z

Elbers, J.P., Taylor, S.S., 2016. Major histocompatibility complex polymorphism in reptile conservation. Herpetol. Conserv. Biol. 11, 1–12.

https://doi.org/10.1146/annurev.genom.1.1.117

Ellis, V.A., Sari, E.H.R., Rubenstein, D.R., Dickerson, R.C., Bensch, S., Ricklefs, R.E., 2018. The global biogeography of avian haemosporidian parasites is characterized by local diversification and intercontinental dispersal. Parasitology 1–7. https://doi.org/10.1017/S0031182018001130

Elsen, P.R., Tingley, M.W., 2015. Global mountain topography and the fate of montane species under climate change. Nat. Clim. Chang. 5, 5–10. https://doi.org/10.1038/nclimate2656

Elton, C.S., 1958. The Ecology of Invasions by Animals and Plants, The Ecology of Invasions by Animals and Plants. Springer US. https://doi.org/10.1007/978-1-4899-7214-9

Enard, D., Cai, L., Gwennap, C., Petrov, D.A., 2016. Viruses are a dominant driver of protein adaptation in mammals. Elife 5, e12469. https://doi.org/10.7554/eLife.12469

Epstein, B., Jones, M., Hamede, R., Hendricks, S., McCallum, H., Murchison, E.P., Schönfeld, B., Wiench, C., Hohenlohe, P., Storfer, A., 2016. Rapid evolutionary response to a transmissible cancer in Tasmanian devils. Nat. Commun. 7, 12684. https://doi.org/10.1038/ncomms12684

Eskew, E.A., Shock, B.C., Ladouceur, E.E.B., Keel, K., Miller, M.R., Foley, J.E., Todd, B.D., 2018. Gene expression differs in susceptible and resistant amphibians exposed to batrachochytrium dendrobatidis. R. Soc. Open Sci. 5, 170910. https://doi.org/10.1098/rsos.170910

Eszterbauer, E., Forró, B., Tolnai, Z., Guti, C.F., Zsigmond, G., Hoitsy, G., Kallert, D.M., 2015. Parental genetic diversity of brown trout (Salmo trutta m. fario) brood stock affects offspring susceptibility to whirling disease. Parasites and Vectors 8, 1–9.

https://doi.org/10.1186/s13071-015-0744-2

Ewald, P.W., 1987. Transmission Modes and Evolution of the Parasitism-Mutualism Continuum. Ann. N. Y. Acad. Sci. 503, 295–306. https://doi.org/10.1111/j.1749-6632.1987.tb40616.x

Ewen, J.G., Bensch, S., Blackburn, T.M., Bonneaud, C., Brown, R., Cassey, P., Clarke, R.H., Pérez-Tris, J., 2012. Establishment of exotic parasites: The origins and characteristics of an avian malaria community in an isolated island avifauna. Ecol. Lett. 15, 1112–1119. https://doi.org/10.1111/j.1461-0248.2012.01833.x

Ezenwa, V.O., Ghai, R.R., McKay, A.F., Williams, A.E., 2016. Group living and pathogen infection revisited. Curr. Opin. Behav. Sci. 12, 66–72. https://doi.org/10.1016/j.cobeha.2016.09.006

Faircloth, B.C., 2016. PHYLUCE is a software package for the analysis of conserved genomic loci. Bioinformatics 32, 786–788. https://doi.org/10.1093/bioinformatics/btv646

Faircloth, B.C., McCormack, J.E., Crawford, N.G., Harvey, M.G., Brumfield, R.T., Glenn, T.C., 2012. Ultraconserved elements anchor thousands of genetic markers spanning multiple evolutionary timescales. Syst. Biol. 61, 717–726. https://doi.org/10.1093/sysbio/sys004

Faith, D.P., 2008. Threatened Species and the Potential Loss of Phylogenetic Diversity: Conservation Scenarios Based on Estimated Extinction Probabilities and Phylogenetic Risk Analysis. Conserv. Biol. 22, 1461–1470.

Fallon, S.M., Bermingham, E., Ricklefs, R.E., 2005. Host Specialization and Geographic Localization of Avian Malaria Parasites: A Regional Analysis in the Lesser Antilles. Am. Nat. 165, 466–480. https://doi.org/10.1086/428430

Faria, N.R., Suchard, M.A., Rambaut, A., Lemey, P., 2011. Toward a quantitative understanding

of viral phylogeography. Curr. Opin. Virol. 1, 423–429. https://doi.org/10.1016/j.coviro.2011.10.003

Fecchio, A., Bell, J.A., Collins, M.D., Farias, I.P., Trisos, C.H., Tobias, J.A., Tkach, V.V., Weckstein, J.D., Ricklefs, R.E., Batalha-Filho, H., 2018. Diversification by host switching and dispersal shaped the diversity and distribution of avian malaria parasites in Amazonia. Oikos 127, 1233–1242. https://doi.org/10.1111/oik.05115

Fecchio, A., Bell, J.A., Pinheiro, R.B.P., Cueto, V.R., Gorosito, C.A., Lutz, H.L., Gaiotti, M.G., Paiva, L. V., França, L.F., Toledo-Lima, G., Tolentino, M., Pinho, J.B., Tkach, V. V., Fontana, C.S., Grande, J.M., Santillán, M.A., Caparroz, R., Roos, A.L., Bessa, R., Nogueira, W., Moura, T., Nolasco, E.C., Comiche, K.J.M., Kirchgatter, K., Guimarães, L.O., Dispoto, J.H., Marini, M.Â., Weckstein, J.D., Batalha-Filho, H., Collins, M.D., 2019a. Avian host composition, local speciation, and dispersal drive the regional assembly of avian malaria parasites in South American birds. Mol. Ecol. https://doi.org/10.1111/mec.15094

Fecchio, A., Lima, M.R., Silveira, P., Braga, É.M., Marini, M.Â., 2011. High prevalence of blood parasites in social birds from a neotropical savanna in Brazil. Emu 111, 132. https://doi.org/10.1071/MU10063

Fecchio, A., Pinheiro, R., Felix, G., Faria, I.P., Pinho, J.B., Lacorte, G.A., Braga, E.M., Farias, I.P., Aleixo, A., Tkach, V. V., Collins, M.D., Bell, J.A., Weckstein, J.D., 2017. Host community similarity and geography shape the diversity and distribution of haemosporidian parasites in Amazonian birds. Ecography (Cop.). 41, 505–515. https://doi.org/10.1111/ecog.03058

Fecchio, A., Wells, K., Bell, J.A., Tkach, V. V, Lutz, H.L., Weckstein, J.D., Clegg, S.M., Clark,

N.J., 2019b. Climate variation influences host specificity in avian malaria parasites. Ecol. Lett. 22, 547–557. https://doi.org/10.1111/ele.13215

Field, K.A., Johnson, J.S., Lilley, T.M., Reeder, S.M., Rogers, E.J., Behr, M.J., Reeder, D.A.M., 2015. The White-Nose Syndrome Transcriptome: Activation of Anti-fungal Host Responses in Wing Tissue of Hibernating Little Brown Myotis. PLoS Pathog. 11, 1–29. https://doi.org/10.1371/journal.ppat.1005168

Fisher, M.C., Henk, D.A., Briggs, C.J., Brownstein, J.S., Madoff, L.C., McCraw, S.L., Gurr, S.J., 2012. Emerging fungal threats to animal, plant and ecosystem health. Nature 484, 1–18. https://doi.org/10.1038/nature10947

Flaherty, B.R., Talundzic, E., Barratt, J., Kines, K.J., Olsen, C., Lane, M., Sheth, M., Bradbury, R.S., 2018. Restriction enzyme digestion of host DNA enhances universal detection of parasitic pathogens in blood via targeted amplicon deep sequencing. Microbiome 6, 164. https://doi.org/10.1186/s40168-018-0540-2

Flint, P.L., Franson, J.C., 2009. Does influenza A affect body condition of wild mallard ducks, or vice versa? Proc. R. Soc. B Biol. Sci. 276, 2345–2346. https://doi.org/10.1098/rspb.2008.1962

Fortini, L.B., Vorsino, A.E., Amidon, F.A., Paxton, E.H., Jacobi, J.D., 2015. Large-scale range collapse of hawaiian forest birds under climate change and the need 21st century conservation options. PLoS One 10, 1–22. https://doi.org/10.1371/journal.pone.0140389

Frank, S.A., 1996. Models of Parasite Virulence. Q. Rev. Biol. 71, 37–78.

Freeman, B.G., Class Freeman, A.M., 2014. Rapid upslope shifts in New Guinean birds illustrate strong distributional responses of tropical montane species to global warming. Proc. Natl.

Acad. Sci. 111, 4490–4. https://doi.org/10.1073/pnas.1318190111

Frick, W.F., Pollock, J.F., Hicks, A.C., Langwig, K.E., Reynolds, D.S., Turner, G.G., Butchkoski, C.M., Kunz, T.H., 2010. An Emerging Disease Causes Regional Population Collapse of a Common North American Bat Species. Science 329, 679–682. https://doi.org/10.1126/science.1188836

Fu, M., Waldman, B., 2017. Major histocompatibility complex variation and the evolution of resistance to amphibian chytridiomycosis. Immunogenetics 69, 529–536. https://doi.org/10.1007/s00251-017-1008-4

Galen, S.C., Borner, J., Martinsen, E.S., Schaer, J., Austin, C.C., West, C.J., Perkins, S.L., 2018. The polyphyly of Plasmodium: Comprehensive phylogenetic analyses of the malaria parasites (Order Haemosporida) reveal widespread taxonomic conflict. R. Soc. Open Sci. 5. https://doi.org/10.1098/rsos.171780

Galen, S.C., Borner, J., Williamson, J.L., Witt, C.C., Perkins, S.L., 2019. Metatranscriptomics yields new genomic resources and sensitive detection of infections for diverse blood parasites, Molecular Ecology Resources. https://doi.org/10.1111/1755-0998.13091

Garamszegi, L., 2014. Modern phylogenetic comparative methods and their application in evolutionary biology: concepts and practice. Springer. https://doi.org/10.1007/978-3-662-43550-2_5

Garamszegi, Z., 2011. Climate change increases the risk of malaria in birds 1751–1759. https://doi.org/10.1111/j.1365-2486.2010.02346.x

Gardner, M.J., Hall, N., Fung, E., White, O., Berriman, M., Hyman, R.W., Carlton, J.M., Pain, A., Nelson, K.E., Bowman, S., Paulsen, I.T., James, K., Eisen, J.A., Rutherford, K.,

Salzberg, S.L., Craig, A., Kyes, S., Chan, M.S., Nene, V., Shallom, S.J., Suh, B., Peterson, J., Angiuoli, S., Pertea, M., Allen, J., Selengut, J., Haft, D., Mather, M.W., Vaidya, A.B., Martin, D.M.A., Fairlamb, A.H., Fraunholz, M.J., Roos, D.S., Ralph, S.A., McFadden, G.I., Cummings, L.M., Subramanian, G.M., Mungall, C., Venter, J.C., Carucci, D.J., Hoffman, S.L., Davis, R.W., Fraser, C.M., Barrell, B., 2002. Genome sequence of the human malaria parasite Plasmodium falciparum. Nature 419, 498–511. https://doi.org/10.1038/nature01097

Gariepy, T.D., Lindsay, R., Ogden, N., Gregory, T.R., 2012. Identifying the last supper: Utility of the DNA barcode library for bloodmeal identification in ticks. Mol. Ecol. Resour. 12, 646–652. https://doi.org/10.1111/j.1755-0998.2012.03140.x

Garnham, P.C.C., 1966. Malaria Parasites and other Haemosporidia., Malaria Parasites and other Haemosporidia. Blackwell Scientific Publications Ltd.

Garvin, M.C., Greiner, E.C., 2003. Ecology of culicoides (Diptera: Ceratopogonidae) in southcentral florida and experimental culicoides vectors of the avian hematozoan Haemoproteus danilewskyi kruse. J. Wildl. Dis. 39, 170–178.

Garvin, M.C., Remsen, J. V, 1997. An alternative hypothesis for heavier parasite loads of brightly colored birds:Exposure at the nest. Auk 114, 179–191.

Ghilarov, A.M., 2000. Ecosystem functioning and intrinsic value of biodiversity. Oikos 90, 408–412. https://doi.org/10.1034/j.1600-0706.2000.900222.x

Gilbert, J.A., Jansson, J.K., Knight, R., 2014. The Earth Microbiome project: Successes and aspirations. BMC Biol. 12, 1–4. https://doi.org/10.1186/s12915-014-0069-1

Gilbertson, M.L.J., Fountain-Jones, N.M., Craft, M.E., 2018. Incorporating genomic methods into contact networks to reveal new insights into animal behaviour and infectious disease

dynamics. Behaviour 155, 759–791. https://doi.org/10.1163/1568539X-00003471

Gire, S.K., Goba, A., Andersen, K.G., Sealfon, R.S.G., Park, D.J., Kanneh, L., Jalloh, S., Momoh, M., Fullah, M., Dudas, G., Wohl, S., Moses, L.M., Yozwiak, N.L., Winnicki, S., Matranga, C.B., Malboeuf, C.M., Qu, J., Gladden, A.D., Schaffner, S.F., Yang, X., Jiang, P.P., Nekoui, M., Colubri, A., Coomber, M.R., Fonnie, M., Moigboi, A., Gbakie, M., Kamara, F.K., Tucker, V., Konuwa, E., Saffa, S., Sellu, J., Jalloh, A.A., Kovoma, A., Koninga, J., Mustapha, I., Kargbo, K., Foday, M., Yillah, M., Kanneh, F., Robert, W., Massally, J.L.B., Chapman, S.B., Bochicchio, J., Murphy, C., Nusbaum, C., Young, S., Birren, B.W., Grant, D.S., Scheiffelin, J.S., Lander, E.S., Happi, C., Gevao, S.M., Gnirke, A., Rambaut, A., Garry, R.F., Khan, S.H., Sabeti, P.C., 2014. Genomic surveillance elucidates Ebola virus origin and transmission during the 2014 outbreak. Science 345, 1369–1372. https://doi.org/10.1126/science.1259657

Glenn, T.C., Nilsen, R.A., Kieran, T.J., Sanders, J.G., Bayona-Vásquez, N.J., Finger, J.W., Pierson, T.W., Bentley, K.E., Hoffberg, S.L., Louha, S., Garcia-De Leon, F.J., Del Rio Portilla, M.A., Reed, K.D., Anderson, J.L., Meece, J.K., Aggrey, S.E., Rekaya, R., Alabady, M., Belanger, M., Winker, K., Faircloth, B.C., 2019. Adapterama I: Universal stubs and primers for 384 unique dual-indexed or 147,456 combinatorially-indexed Illumina libraries (iTru & iNext). PeerJ. https://doi.org/10.7717/peerj.7755

González, A.D., Matta, N.E., Ellis, V.A., Miller, E.T., Ricklefs, R.E., Gutiérrez, H.R., 2014. Mixed species flock, nest height, and elevation partially explain avian haemoparasite prevalence in Colombia. PLoS One 9. https://doi.org/10.1371/journal.pone.0100695

Gopinath, S., Lichtman, J.S., Bouley, D.M., Elias, J.E., Monack, D.M., 2014. Role of disease-

associated tolerance in infectious superspreaders. Proc. Natl. Acad. Sci. 111, 15780–15785. https://doi.org/10.1073/pnas.1409968111

Goslee, S., Urban, D., 2015. Package ‘ ecodist .’ R Top. Doc.

Goudet, J., 2005. HIERFSTAT, a package for R to compute and test hierarchical F-statistics. Mol. Ecol. Notes. https://doi.org/10.1111/j.1471-8286.2004.00828.x

Gower, J.C., 1971. A General Coefficient of Similarity and Some of Its Properties. Biometrics 27, 857–871. https://doi.org/10.2307/2528823

Grenfell, B.T., Pybus, O.G., Gog, J.R., Wood, J.L.N., Daly, J.M., Mumford, J.A., Holmes, E.C., 2004. Unifying the Epidemiological and Evolutionary Dynamics of Pathogens. Science 303, 327–333. https://doi.org/10.1126/science.1090727

Griffin, R.H., Nunn, C.L., 2012. Community structure and the spread of infectious disease in primate social networks. Evol. Ecol. 26, 779–800. https://doi.org/10.1007/s10682-011-9526-2

Grogan, L.F., Berger, L., Rose, K., Grillo, V., Cashins, S.D., Skerratt, L.F., 2014. Surveillance for Emerging Biodiversity Diseases of Wildlife. PLoS Pathog. 10. https://doi.org/10.1371/journal.ppat.1004015

Gubler, D.J., 1998. Resurgent vector-borne diseases as a global health problem. Emerg. Infect. Dis. 4, 442–50. https://doi.org/10.3201/eid0403.980326

Gupta, P., Vishnudas, C.K., Ramakrishnan, U., Robin, V. V, Dharmarajan, G., 2019. Geographical and host species barriers differentially affect generalist and specialist parasite community structure in a tropical sky-island archipelago. Proc. R. Soc. B 286.

Gupta, R., 1960. Ecological notes on the vegetation of Kodaikanal in South India. J. Indian Bot.

Soc. 39, 601–607.

Hadfield, J.D., 2010. MCMC methods for multi-response generalized linear mixed models: the MCMCglmm R package. J. Stat. Softw. 33, 1–22. https://doi.org/10.1002/ana.22635

Hadfield, J.D., Nakagawa, S., 2010. General quantitative genetic methods for comparative biology: Phylogenies, taxonomies and multi-trait models for continuous and categorical characters. J. Evol. Biol. 23, 494–508. https://doi.org/10.1111/j.1420-9101.2009.01915.x

Hall, N., Karras, M., Raine, J.D., Carlton, J.M., Kooij, T.W.A., Berriman, M., Florens, L., Janssen, C.S., Pain, A., Christophides, G.K., James, K., Rutherford, K., Harris, B., Harris, D., Churcher, C., Quail, M.A., Ormond, D., Doggett, J., Trueman, H.E., Mendoza, J., Bidwell, S.L., Rajandream, M.A., Carucci, D.J., Yates, J.R., Kafatos, F.C., Janse, C.J., Barrell, B., Turner, C.M.R., Waters, A.P., Sinden, R.E., 2005. A comprehensive survey of the Plasmodium life cycle by genomic, transcriptomic, and proteomic analyses. Science. https://doi.org/10.1126/science.1103717

Hamer, G.L., Kitron, U.D., Goldberg, T.L., Brawn, J.D., Loss, S.R., Ruiz, M.O., Hayes, D.B., Walker, E.D., 2009. Host selection by Culex pipiens mosquitoes and west nile virus amplification. Am. J. Trop. Med. Hyg. 80, 268–278. https://doi.org/80/2/268 [pii]

Hamilton, W.D., Zuk, M., 1982. Heritable True Fitness and Bright Birds : A Role for Parasites? Science 218, 384–387.

Harrigan, R.J., Sedano, R., Chasar, A.C., Chaves, J.A., Nguyen, J.T., Whitaker, A., Smith, T.B., 2014. New host and lineage diversity of avian haemosporidia in the northern Andes. Evol. Appl. 7, 799–811. https://doi.org/10.1111/eva.12176

Harris, R.N., Brucker, R.M., Walke, J.B., Becker, M.H., Schwantes, C.R., Flaherty, D.C., Lam,

B.A., Woodhams, D.C., Briggs, C.J., Vredenburg, V.T., Minbiole, K.P.C.C., 2009. Skin microbes on frogs prevent morbidity and mortality caused by a lethal skin fungus. ISME J. 3, 818–824. https://doi.org/10.1038/ismej.2009.27

Harvell, C.D., Mitchell, C.E., Ward, J.R., Altizer, S., Dobson, A.P., Ostfeld, R.S., Samuel, M.D., 2002. Climate warming and disease risks for terrestrial and marine biota. Science 296, 2158–62. https://doi.org/10.1126/science.1063699

Hatcher, M.J., Dick, J.T., Dunn, A.M., 2012a. Diverse effects of parasites in ecosystems: linking interdependent processes. Front. Ecol. Environ. 10, 186–194. https://doi.org/10.1890/110016

Hatcher, M.J., Dick, J.T.A., Dunn, A.M., 2014. Parasites that change predator or prey behaviour can have keystone effects on community composition. Biol. Lett. 10, 0–4. https://doi.org/10.1098/rsbl.2013.0879

Hatcher, M.J., Dick, J.T.A., Dunn, A.M., 2012b. Disease emergence and invasions. Funct. Ecol. 26, 1275–1287. https://doi.org/10.1111/j.1365-2435.2012.02031.x

Hawley, D.M., Altizer, S.M., 2011. Disease ecology meets ecological immunology: Understanding the links between organismal immunity and infection dynamics in natural populations. Funct. Ecol. 25, 48–60. https://doi.org/10.1111/j.1365-2435.2010.01753.x

Hayakawa, T., Culleton, R., Otani, H., Horii, T., Tanabe, K., 2008. Big bang in the evolution of extant malaria parasites. Mol. Biol. Evol. 25, 2233–2239. https://doi.org/10.1093/molbev/msn171

Hedrick, P.W., Kim, T.J., Parker, K.M., 2001. Parasite resistance and genetic variation in the endangered Gila topminnow. Anim. Conserv. 4, 103–109.

https://doi.org/10.1017/S1367943001001135

Hellgren, O., Bensch, S., Malmqvist, B., 2008. Bird hosts, blood parasites and their vectors - Associations uncovered by molecular analyses of blackfly blood meals. Mol. Ecol. 17, 1605–1613. https://doi.org/10.1111/j.1365-294X.2007.03680.x

Hellgren, O., Križanauskienė, A., Hasselquist, D., Bensch, S., 2011. Low haemosporidian diversity and one key-host species in a bird malaria community on a mid-Atlantic island (São Miguel, Azores). J. Wildl. Dis. 47, 849–59. https://doi.org/10.7589/0090-3558-47.4.849

Hellgren, O., Waldenström, J., Bensch, S., Heligren, O., Waldenstrom, J., Bensch, S., Hellgren, O., Waldenström, J., Bensch, S., 2004. A new PCR assay for simultaneous studies of Leucocytozoan, Plasmodium, and Haemoproteus from avian blood. J. Parasitol. 90, 797–802. https://doi.org/10.1645/GE-184R1

Heloise, C., Garant, D., Robert, K., Mainguy, J., Pelletier, F., 2012. Genetic structure and rabies spread potential in raccoons: The role of landscape barriers and sex-biased dispersal. Evol. Appl. 5, 393–404. https://doi.org/10.1111/j.1752-4571.2012.00238.x

Hernandez, A.D., Sukhdeo, M.V.K., 2008. Parasites alter the topology of a stream food web across seasons. Oecologia 156, 613–624. https://doi.org/10.1007/s00442-008-0999-9

Herrera, J., Nunn, C.L., 2019. Behavioural ecology and infectious disease: Implications for conservation of biodiversity. Philos. Trans. R. Soc. B Biol. Sci. https://doi.org/10.1098/rstb.2018.0054

Hess, A.D., Hayes, R.O., 1970. Relative potentials of domestic animals for zooprophylaxis against mosquito vectors of encephalitis. Am. J. Trop. Med. Hyg. 19, 327–334.

https://doi.org/10.4269/ajtmh.1970.19.327

Hilker, F.M., Lewis, M.A., Seno, H., Langlais, M., Malchow, H., 2005. Pathogens can slow down or reverse invasion fronts of their hosts. Biol. Invasions 7, 817–832. https://doi.org/10.1007/s10530-005-5215-9

Hill, N.J., Takekawa, J.Y., Ackerman, J.T., Hobson, K.A., Herring, G., Cardona, C.J., Runstadler, J.A., Boyce, W.M., 2012a. Migration strategy affects avian influenza dynamics in mallards (Anas platyrhynchos). Mol. Ecol. 21, 5986–5999. https://doi.org/10.1111/j.1365-294X.2012.05735.x

Hill, N.J., Takekawa, J.Y., Cardona, C.J., Meixell, B.W., Ackerman, J.T., Runstadler, J.A., Boyce, W.M., 2012b. Cross-Seasonal Patterns of Avian Influenza Virus in Breeding and Wintering Migratory Birds: A Flyway Perspective. Vector-Borne Zoonotic Dis. 12, 243–253. https://doi.org/10.1089/vbz.2010.0246

Hindson, B.J., Ness, K.D., Masquelier, D.A., Belgrader, P., Heredia, N.J., Makarewicz, A.J., Bright, I.J., Lucero, M.Y., Hiddessen, A.L., Legler, T.C., Kitano, T.K., Hodel, M.R., Petersen, J.F., Wyatt, P.W., Steenblock, E.R., Shah, P.H., Bousse, L.J., Troup, C.B., Mellen, J.C., Wittmann, D.K., Erndt, N.G., Cauley, T.H., Koehler, R.T., So, A.P., Dube, S., Rose, K.A., Montesclaros, L., Wang, S., Stumbo, D.P., Hodges, S.P., Romine, S., Milanovich, F.P., White, H.E., Regan, J.F., Karlin-Neumann, G.A., Hindson, C.M., Saxonov, S., Colston, B.W., 2011. High-throughput droplet digital PCR system for absolute quantitation of DNA copy number. Anal. Chem. 83, 8604–8610. https://doi.org/10.1021/ac202028g

Hoberg, E.P., Brooks, D.R., 2015. Evolution in action: climate change, biodiversity dynamics

and emerging infectious disease. Philos. Trans. R. Soc. B Biol. Sci. 370, 20130553. https://doi.org/10.1098/rstb.2013.0553

Holt, E.A., Miller, S.W., 2011. Bioindicators: using organisms to measure environmental impacts. Nat. Educ. Knowl. 3, 8.

Hooper, L. V., Littman, D.R., Macpherson, A.J., 2012. Interactions between the microbiota and the immune system. Science 336, 1268–1273. https://doi.org/10.1126/science.1223490

Horwitz, P., Wilcox, B.A., 2005. Parasites, ecosystems and sustainability: An ecological and complex systems perspective. Int. J. Parasitol. 35, 725–732. https://doi.org/10.1016/j.ijpara.2005.03.002

Howe, L., Castro, I.C., Schoener, E.R., Hunter, S., Barraclough, R.K., Alley, M.R., 2012. Malaria parasites (Plasmodium spp.) infecting introduced, native and endemic New Zealand birds. Parasitol. Res. 110, 913–23. https://doi.org/10.1007/s00436-011-2577-z

Hoyt, J.R., Cheng, T.L., Langwig, K.E., Hee, M.M., Frick, W.F., Kilpatrick, A.M., 2015. Bacteria Isolated from Bats Inhibit the Growth of Pseudogymnoascus destructans, the Causative Agent of White-Nose Syndrome. PLoS One 10, e0121329. https://doi.org/10.1371/journal.pone.0121329

Hoyt, J.R., Langwig, K.E., White, J.P., Kaarakka, H.M., Redell, J.A., Parise, K.L., Frick, W.F., Foster, J.T., Kilpatrick, A.M., 2019. Field trial of a probiotic bacteria to protect bats from white-nose syndrome. Sci. Rep. 9, 1–9. https://doi.org/10.1038/s41598-019-45453-z

Huang, X., Hansson, R., Palinauskas, V., Valkiūnas, G., Hellgren, O., Bensch, S., Valki Unas B, G., Hellgren, O., Bensch, S., 2018. The success of sequence capture in relation to phylogenetic distance from a reference genome: a case study of avian haemosporidian

parasites. Int. J. Parasitol. 48, 947–954. https://doi.org/10.1016/j.ijpara.2018.05.009

Hudson, P.J., Dobson, A.P., Lafferty, K.D., 2006. Is a healthy ecosystem one that is rich in parasites? Trends Ecol. Evol. 21, 381–385. https://doi.org/10.1016/j.tree.2006.04.007

Huggins, L.G., Koehler, A. V., Ng-Nguyen, D., Wilcox, S., Schunack, B., Inpankaew, T., Traub, R.J., 2019. A novel metabarcoding diagnostic tool to explore protozoan haemoparasite diversity in mammals: a proof-of-concept study using canines from the tropics. Sci. Rep. 9, 1–10. https://doi.org/10.1038/s41598-019-49118-9

Hughes, A.L., 1991. Society for Conservation Biology MHC Polymorphism and the Design of Captive Breeding Programs. Conserv. Biol. 5, 249–251. https://doi.org/10.1111/j.1523-1739.1991.tb00130.x

Huspeni, T.C., Lafferty, K.D., 2004. Using larval trematodes that parasitize snails to evaluate a saltmarsh restoration project. Ecol. Appl. 14, 795–804. https://doi.org/10.1890/01-5346

Huver, J.R., Koprivnikar, J., Johnson, P.T.J., Whyard, S., 2015. Development and application of an eDNA method to detect and quantify a pathogenic parasite in aquatic ecosystems. Ecol. Appl. 25, 991–1002. https://doi.org/10.1890/14-1530.1

Illera, J.C., Padilla, L.G., Lopez, G., Angel, M., 2017. Factors governing the prevalence and richness of avian haemosporidian communities within and between temperate mountains. PLoS One 12, 1–22.

Isaksson, C., Sepil, I., Baramidze, V., Sheldon, B.C., 2013. Explaining variance of avian malaria infection in the wild : the importance of host density , habitat , individual life-history and oxidative stress. BMC Ecol. 13, 15. https://doi.org/10.1186/1472-6785-13-15

Ishtiaq, F., Bowden, C.G.R., Jhala, Y. V., 2017. Seasonal dynamics in mosquito abundance and

temperature do not influence avian malaria prevalence in the Himalayan foothills. Ecol. Evol. 8040–8057. https://doi.org/10.1002/ece3.3319

Ishtiaq, F., Gering, E., Rappole, J.H., Rahmani, A.R., Jhala, Y. V., Dove, C.J., Milensky, C., Olson, S.L., Peirce, M.A., Fleischer, R.C., 2007. Prevalence and diversity of avian hematozoan parasites in asia : a regional survey. J. Wildl. Dis. 43, 382–398. https://doi.org/10.7589/0090-3558-43.3.382

James, T.Y., Litvintseva, A.P., Vilgalys, R., Morgan, J.A.T., Taylor, J.W., Fisher, M.C., Berger, L., Weldon, C., Du Preez, L., Longcore, J.E., 2009. Rapid global expansion of the fungal disease chytridiomycosis into declining and healthy amphibian populations. PLoS Pathog. 5, e1000458. https://doi.org/10.1371/journal.ppat.1000458

Jiang, T., Shwab, E.K., Martin, R.M., Gerhold, R.W., Rosenthal, B.M., Dubey, J.P., Su, C., 2018. A partition of Toxoplasma gondii genotypes across spatial gradients and among host species, and decreased parasite diversity towards areas of human settlement in North America. Int. J. Parasitol. 48, 611–619. https://doi.org/10.1016/j.ijpara.2018.01.008

Johnson, P.T.J., de Roode, J.C., Fenton, A., 2015a. Why infectious disease research needs community ecology. Science 349, 1259504. https://doi.org/10.1126/science.1259504

Johnson, P.T.J., Dobson, A., Lafferty, K.D., Marcogliese, D.J., Memmott, J., Orlofske, S.A., Poulin, R., Thieltges, D.W., 2010. When parasites become prey: Ecological and epidemiological significance of eating parasites. Trends Ecol. Evol. 25, 362–371. https://doi.org/10.1016/j.tree.2010.01.005

Johnson, P.T.J., Ostfeld, R.S., Keesing, F., 2015b. Frontiers in research on biodiversity and disease. Ecol. Lett. 18, 1119–1133. https://doi.org/10.1111/ele.12479

Johnson, P.T.J., Preston, D.L., Hoverman, J.T., Richgels, K.L.D., 2013. Biodiversity decreases disease through predictable changes in host community competence. Nature 494, 230–233. https://doi.org/10.1038/nature11883

Johnson, P.T.J., Rohr, J.R., Hoverman, J.T., Kellermanns, E., Bowerman, J., Lunde, K.B., 2012. Living fast and dying of infection: Host life history drives interspecific variation in infection and disease risk. Ecol. Lett. 15, 235–242. https://doi.org/10.1111/j.1461-0248.2011.01730.x

Jones, K.E., Patel, N.G., Levy, M.A., Storeygard, A., Balk, D., Gittleman, J.L., Daszak, P., 2008. Global trends in emerging infectious diseases. Nature 451, 990–994. https://doi.org/10.1038/nature06536

Jones, M.R., Good, J.M., 2016. Targeted capture in evolutionary and ecological genomics. Mol. Ecol. 25, 185–202. https://doi.org/10.1111/mec.13304

Joshi, A.A., Ratnam, J., Sankaran, M., 2019. Frost maintains forests and grasslands as alternate states in a montane tropical forest–grassland mosaic; but alien tree invasion and warming can disrupt this balance. J. Ecol. 122–132. https://doi.org/10.1111/1365-2745.13239

Joshi, J., Karanth, P., 2013. Did southern Western Ghats of peninsular India serve as refugia for its endemic biota during the Cretaceous volcanism? Ecol. Evol. 3, 3275–3282. https://doi.org/10.1002/ece3.603

Justus, J., Colyvan, M., Regan, H., Maguire, L., 2009. Buying into conservation: intrinsic versus instrumental value. Trends Ecol. Evol. 24, 187–191. https://doi.org/10.1016/j.tree.2008.11.011

Kamath, P.L., Foster, J.T., Drees, K.P., Luikart, G., Quance, C., Anderson, N.J., Clarke, P.R.,

Cole, E.K., Drew, M.L., Edwards, W.H., Rhyan, J.C., Treanor, J.J., Wallen, R.L., White, P.J., Robbe-Austerman, S., Cross, P.C., 2016. Genomics reveals historic and contemporary transmission dynamics of a bacterial disease among wildlife and livestock. Nat. Commun. 7, 1–10. https://doi.org/10.1038/ncomms11448

Kamiya, T., O'Dwyer, K., Westerdahl, H., Senior, A., Nakagawa, S., 2014. A quantitative review of MHC-based mating preference: The role of diversity and dissimilarity. Mol. Ecol. 23, 5151–5163. https://doi.org/10.1111/mec.12934

Kamoroff, C., Goldberg, C.S., 2017. Using environmental DNA for early detection of amphibian chytrid fungus Batrachochytrium dendrobatidis prior to a ranid die-off. Dis. Aquat. Organ. 127, 75–79. https://doi.org/10.3354/dao03183

Kao, R.R., Haydon, D.T., Lycett, S.J., Murcia, P.R., 2014. Supersize me: How whole-genome sequencing and big data are transforming epidemiology. Trends Microbiol. 22, 282–291. https://doi.org/10.1016/j.tim.2014.02.011

Kapusta, A., Suh, A., Feschotte, C., 2017. Dynamics of genome size evolution in birds and mammals. Proc. Natl. Acad. Sci. 114, E1460–E1469. https://doi.org/10.1073/pnas.1616702114

Karanth, K.P., 2003. Evolution of disjunct distributions among wet-zone species of the Indian subcontinent: Testing various hypotheses using a phylogenetic approach. Curr. Sci. 85, 1276–1283.

Katoh, K., Standley, D.M., 2013. MAFFT multiple sequence alignment software version 7: Improvements in performance and usability. Mol. Biol. Evol. 30, 772–780. https://doi.org/10.1093/molbev/mst010

Kauffman, E.B., Jones, S.A., Dupuis, A.P., Ngo, K.A., Bernard, K.A., Kramer, L.D., 2003. Virus detection protocols for West Nile virus in vertebrate and mosquito specimens. J. Clin. Microbiol. 41, 3661–3667. https://doi.org/10.1128/JCM.41.8.3661-3667.2003

Keane, R.M., Crawley, M.J., 2002. Exotic plant invasions and the enemy release hypothesis. Trends Ecol. Evol. 17, 164–170. https://doi.org/10.1016/S0169-5347(02)02499-0

Kearse, M., Moir, R., Wilson, A., Stones-Havas, S., Cheung, M., Sturrock, S., Buxton, S., Cooper, A., Markowitz, S., Duran, C., Thierer, T., Ashton, B., Meintjes, P., Drummond, A., 2012. Geneious Basic: An integrated and extendable desktop software platform for the organization and analysis of sequence data. Bioinformatics 28, 1647–1649. https://doi.org/10.1093/bioinformatics/bts199

Keesing, F., Ostfeld, R.S., 2012. Disease ecology, in: Ingram, J.C., DeClerck, F., Rio, C.R. del (Eds.), Integrating Ecology and Poverty Reduction: Ecological Dimensions. Springer-Verlag, New York, NY, pp. 217–230. https://doi.org/10.1007/978-1-4419-0633-5_13

Keith, D.A., Rodríguez, J.P., Brooks, T.M., Burgman, M.A., Barrow, E.G., Bland, L., Comer, P.J., Franklin, J., Link, J., Mccarthy, M.A., Miller, R.M., Murray, N.J., Nel, J., Nicholson, E., Oliveira-Miranda, M.A., Regan, T.J., Rodríguez-Clark, K.M., Rouget, M., Spalding, M.D., 2015. The IUCN red list of ecosystems: Motivations, challenges, and applications. Conserv. Lett. 8, 214–226. https://doi.org/10.1111/conl.12167

Kelly, A.C., Mateus-Pinilla, N.E., Brown, W., Ruiz, M.O., Douglas, M.R., Douglas, M.E., Shelton, P., Beissel, T., Novakofski, J., 2014. Genetic assessment of environmental features that influence deer dispersal: Implications for prion-infected populations. Popul. Ecol. 56, 327–340. https://doi.org/10.1007/s10144-013-0427-9

Kelly, D.W., Paterson, R.A., Toensend, C.R., Poulin, R., Tompkins, D.M., 2009. Parasite spillback: A neglected concept in invasion ecology? Ecology 90, 2047–2056. https://doi.org/10.1890/07-1861.1

Kembel, S.W., Cowan, P.D., Helmus, M.R., Cornwell, W.K., Morlon, H., Ackerly, D.D., Blomberg, S.P., Webb, C.O., 2010. Picante: R tools for integrating phylogenies and ecology. Bioinformatics 26, 1463–1464. https://doi.org/10.1093/bioinformatics/btq166

Kilpatrick, A.M., Randolph, S.E., 2012. Drivers, dynamics, and control of emerging vector-borne zoonotic diseases. Lancet 380, 1946–1955. https://doi.org/10.1016/S0140-6736(12)61151-9

King, J.L., Smith, A.D., Mitchell, E.A., Allen, M.S., 2017. Validation of droplet digital PCR for the detection and absolute quantification of Borrelia DNA in Ixodes scapularis ticks. Parasitology 144, 359–367. https://doi.org/10.1017/S0031182016001864

King, K.C., Lively, C.M., 2012. Does genetic diversity limit disease spread in natural host populations. Heredity 109, 199–203. https://doi.org/10.1038/hdy.2012.33

Knutie, S.A., Owen, J.P., Mcnew, S.M., Bartlow, A.W., Arriero, E., Herman, J.M., Diblasi, E., Thompson, M., Koop, J.A.H., Clayton, D.H., 2016. Galapagos mockingbirds tolerate introduced parasites that affect Darwin's finches. Ecology 97, 940–950. https://doi.org/10.1890/15-0119.1

Kocher, A., Gantier, J.-C., Gaborit, P., Zinger, L., Holota, H., Valiere, S., Dusfour, I., Girod, R., Bañuls, A.-L., Murienne, J., 2017. Vector soup: high-throughput identification of Neotropical phlebotomine sand flies using metabarcoding. Mol. Ecol. Resour. 17, 172–182. https://doi.org/10.1111/1755-0998.12556

Kock, R.A., Wambua, J.M., Mwanzia, J., Wamwayi, H., Ndungu, E.K., Barrett, T., Kock, N.D., Rossiter, P.B., 1999. Rinderpest epidemic in wild ruminants in Kenya 1993-97. Vet. Rec. 145, 275–283. https://doi.org/10.1136/vr.145.10.275

Koepfli, C., Nguitragool, W., Hofmann, N.E., Robinson, L.J., Ome-Kaius, M., Sattabongkot, J., Felger, I., Mueller, I., 2016. Sensitive and accurate quantification of human malaria parasites using droplet digital PCR (ddPCR). Sci. Rep. 6, 39183. https://doi.org/10.1038/srep39183

Kozakiewicz, C.P., Burridge, C.P., Funk, W.C., VandeWoude, S., Craft, M.E., Crooks, K.R., Ernest, H.B., Fountain-Jones, N.M., Carver, S., 2018. Pathogens in space: Advancing understanding of pathogen dynamics and disease ecology through landscape genetics. Evol. Appl. 11, 1763–1778. https://doi.org/10.1111/eva.12678

Krasnov, B.R., Shenbrot, G.I., Khokhlova, I.S., Mouillot, D., Poulin, R., 2008. Latitudinal gradients in niche breadth: Empirical evidence from haematophagous ectoparasites. J. Biogeogr. 35, 592–601. https://doi.org/10.1111/j.1365-2699.2007.01800.x

Kuris, A.M., Blaustein, A.R., Alio, J.J., 1980. Hosts as Islands. Am. Nat. 116, 570–586. https://doi.org/10.1086/283647

Kuris, A.M., Hechinger, R.F., Shaw, J.C., Whitney, K.L., Aguirre-Macedo, L., Boch, C.A., Dobson, A.P., Dunham, E.J., Fredensborg, B.L., Huspeni, T.C., Lorda, J., Mababa, L., Mancini, F.T., Mora, A.B., Pickering, M., Talhouk, N.L., Torchin, M.E., Lafferty, K.D., 2008. Ecosystem energetic implications of parasite and free-living biomass in three estuaries. Nature 454, 515–518. https://doi.org/10.1038/nature06970

Kutzer, M.A.M., Armitage, S.A.O., 2016. Maximising fitness in the face of parasites : a review

of host tolerance &. Zoology 119, 281–289. https://doi.org/10.1016/j.zool.2016.05.011

La Sorte, F.A., Jetz, W., 2010. Projected range contractions of montane biodiversity under global warming. Proc. Biol. Sci. 277, 3401–10. https://doi.org/10.1098/rspb.2010.0612

Lachish, S., Knowles, S.C.L., Alves, R., Wood, M.J., Sheldon, B.C., 2011. Fitness effects of endemic malaria infections in a wild bird population: The importance of ecological structure. J. Anim. Ecol. 80, 1196–1206. https://doi.org/10.1111/j.1365-2656.2011.01836.x

LaDeau, S.L., Kilpatrick, A.M., Marra, P.P., 2007. West Nile virus emergence and large-scale declines of North American bird populations. Nature 447, 710–713. https://doi.org/10.1038/nature05829

Ladle, R.J., 1992. Parasites and Sex : Catching the Red Queen. Trends Ecol. Evol. 7, 405–408. https://doi.org/10.1016/0169-5347(92)90021-3

Lafferty, K.D., 2009. The ecology of climate change and infectious diseases. Ecology 89, 2712–2724. https://doi.org/10.1890/07-1861.1

Lafferty, K.D., 1997. Environmental parasitology: What can parasites tell us about human impacts on the environment? Parasitol. Today 13, 251–255. https://doi.org/10.1016/S0169-4758(97)01072-7

Lafferty, K.D., Dobson, A.P., Kuris, A.M., 2006. Parasites dominate food web links. Proc. Natl. Acad. Sci. 103, 11211–11216. https://doi.org/10.1073/pnas.0604755103

Lapointe, D.A., Atkinson, C.T., Samuel, M.D., 2012. Ecology and conservation biology of avian malaria. Ann. N. Y. Acad. Sci. 1249, 211–226. https://doi.org/10.1111/j.1749-6632.2011.06431.x

LaPointe, D.A., Goff, M.L., Atkinson, C.T., 2010. Thermal constraints to the sporogonic

development and altitudinal distribution of avian malaria Plasmodium relictum in Hawai'i. J. Parasitol. 96, 318–24. https://doi.org/10.1645/GE-2290.1

Laurance, S.G.W., Jones, D., Westcott, D., McKeown, A., Harrington, G., Hilbert, D.W., 2013. Habitat fragmentation and ecological traits influence the prevalence of avian blood parasites in a tropical rainforest landscape. PLoS One 8, e76227. https://doi.org/10.1371/journal.pone.0076227

Lazzaro, B.P., Clark, A.G., 2012. Rapid evolution of innate immune response genes, in: Singh, R.S., Xu, J., Kulathinal, R.J. (Eds.), Rapidly Evolving Genes and Genetic Systems. https://doi.org/10.1093/acprof:oso/9780199642274.003.0020

le Roex, N., Koets, A.P., van Helden, P.D., Hoal, E.G., 2013. Gene Polymorphisms in African Buffalo Associated with Susceptibility to Bovine Tuberculosis Infection. PLoS One 8, 1–6. https://doi.org/10.1371/journal.pone.0064494

Lee, J.S., Mackie, R.S., Harrison, T., Shariat, B., Kind, T., Kehl, T., Löchelt, M., Boucher, C., VandeWoude, S., 2017. Targeted Enrichment for Pathogen Detection and Characterization in Three Felid Species. J. Clin. Microbiol. 55, 1658–1670. https://doi.org/10.1128/JCM.01463-16

Lee, P.S., Sing, K.W., Wilson, J.J., 2015. Reading mammal diversity from flies: The persistence period of amplifiable mammal mtDNA in blowfly guts (*Chrysomya megacephala*) and a new DNA mini-barcode target. PLoS One 10, 1–12. https://doi.org/10.1371/journal.pone.0123871

Lefèvre, T., Lebarbenchon, C., Gauthier-Clerc, M., Missé, D., Poulin, R., Thomas, F., 2009. The ecological significance of manipulative parasites. Trends Ecol. Evol. 24, 41–48.

https://doi.org/10.1016/j.tree.2008.08.007

Lehane, M.J., 2005. The biology of blood-sucking in insects, 2nd ed. Cambridge University Press. https://doi.org/10.1017/CBO9780511610493

Lemmon, A.R., Emme, S.A., Lemmon, E.M., 2012. Anchored hybrid enrichment for massively high-throughput phylogenomics. Syst. Biol. 61, 727–744. https://doi.org/10.1093/sysbio/sys049

Leung, T.L.F., Poulin, R., 2008. Parasitism, commensalism, and mutualism: Exploring the many shades of symbioses. Vie Milieu 58, 107–115.

Leventhal, G.E., Kouyos, R., Stadler, T., von Wyl, V., Yerly, S., Böni, J., Cellerai, C., Klimkait, T., Günthard, H.F., Bonhoeffer, S., 2012. Inferring epidemic contact structure from phylogenetic trees. PLoS Comput. Biol. 8. https://doi.org/10.1371/journal.pcbi.1002413

Levin, I.I., Outlaw, D.C., Vargas, F.H., Parker, P.G., 2009. Plasmodium blood parasite found in endangered Galapagos penguins (*Spheniscus mendiculus*). Biol. Conserv. 142, 3191–3195. https://doi.org/10.1016/j.biocon.2009.06.017

Li, H., Bai, R., Zhao, Z., Tao, L., Ma, M., Ji, Z., Jian, M., Ding, Z., Dai, X., Bao, F., Liu, A., 2018. Application of droplet digital PCR to detect the pathogens of infectious diseases. Biosci. Rep. 38. https://doi.org/10.1042/BSR20181170

Lichstein, J.W., 2007. Multiple regression on distance matrices: A multivariate spatial analysis tool. Plant Ecol. https://doi.org/10.1007/s11258-006-9126-3

Lindström, E.R., Andrén, H., Angelstam, P., Cederlund, G., Jäderberg, L., Lemnell, P., Martinsson, B., Sköld, K., John, E., 1994. Disease Reveals the Predator : Sarcoptic Mange , Red Fox Predation , and Prey Populations. Ecology 75, 1042–1049.

Lipkin, W.I., 2013. The changing face of pathogen discovery and surveillance. Nat. Rev. Microbiol. 11, 133–141. https://doi.org/10.1038/nrmicro2949

Lips, K.R., Brem, F., Brenes, R., Reeve, J.D., Alford, R.A., Voyles, J., Carey, C., Livo, L., Pessier, A.P., Collins, J.P., 2006. Emerging infectious disease and the loss of biodiversity in a Neotropical amphibian community. Proc. Natl. Acad. Sci. 103, 3165–3170. https://doi.org/10.1073/pnas.0506889103

Lively, C.M., Craddock, C., Vrijenhoek, R.C., 1990. Red Queen hypothesis supported by parasitism in sexual and clonal fish. Nature 344, 864–866.

Lively, C.M., Dybdahl, M.F., 2000. Parasite adaptation to locally common host genotypes. Nature 405, 679–681.

LoGiudice, K., Ostfeld, R.S., Schmidt, K.A., Keesing, F., 2003. The ecology of infectious disease: effects of host diversity and community composition on Lyme disease risk. Proc. Natl. Acad. Sci. 100, 567–71. https://doi.org/10.1073/pnas.0233733100

Lorch, J.M., Gargas, A., Meteyer, C.U., Berlowski-Zier, B.M., Green, D.E., Shearn-Bochsler, V., Thomas, N.J., Blehert, D.S., 2010. Rapid polymerase chain reaction diagnosis of white-nose syndrome in bats. J. Vet. Diagnostic Investig. 22, 224–230.

Lovett, G.M., Arthur, M.A., Weathers, K.C., Griffin, J.M., 2010. Long-term Changes in Forest Carbon and Nitrogen Cycling Caused by an Introduced Pest/Pathogen Complex. Ecosystems 13, 1188–1200. https://doi.org/10.1007/s10021-010-9381-y

Luikart, G., Pilgrim, K., Visty, J., Ezenwa, V.O., Schwartz, M.K., 2008. Candidate gene microsatellite variation is associated with parasitism in wild bighorn sheep. Biol. Lett. 4, 228–231. https://doi.org/10.1098/rsbl.2007.0633

Luquet, E., Garner, T.W.J., Léna, J.P., Bruel, C., Joly, P., Lengagne, T., Grolet, O., Plénet, S., 2012. Genetic erosion in wild populations makes resistance to a pathogen more costly. Evolution 66, 1942–1952. https://doi.org/10.1111/j.1558-5646.2011.01570.x

Lutomiah, J., Omondi, D., Masiga, D., Mutai, C., Mireji, P.O., Ongus, J., Linthicum, K.J., Sang, R., 2014. Blood Meal Analysis and Virus Detection in Blood-Fed Mosquitoes Collected During the 2006–2007 Rift Valley Fever Outbreak in Kenya. Vector-Borne Zoonotic Dis. 14, 656–664. https://doi.org/10.1089/vbz.2013.1564

Lutz, H.L., Hochachka, W.M., Engel, J.I., Bell, J.A., Tkach, V. V., Bates, J.M., Hackett, S.J., Weckstein, J.D., 2015. Parasite prevalence corresponds to host life history in a diverse assemblage of afrotropical birds and haemosporidian parasites. PLoS One 10, 1–24. https://doi.org/10.1371/journal.pone.0121254

Lutz, H.L., Marra, N.J., Grewe, F., Carlson, J.S., Palinauskas, V., Valkiūnas, G., Stanhope, M.J., 2016a. Laser capture microdissection microscopy and genome sequencing of the avian malaria parasite, Plasmodium relictum. Parasitol Res 115, 4503–4510. https://doi.org/10.1007/s00436-016-5237-5

Lutz, H.L., Patterson, B.D., Kerbis Peterhans, J.C., Stanley, W.T., Webala, P.W., Gnoske, T.P., Hackett, S.J., Stanhope, M.J., 2016b. Diverse sampling of East African haemosporidians reveals chiropteran origin of malaria parasites in primates and rodents. Mol. Phylogenet. Evol. 99, 7–15. https://doi.org/10.1016/j.ympev.2016.03.004

Lymbery, A.J., Morine, M., Kanani, H.G., Beatty, S.J., Morgan, D.L., 2014. Co-invaders: The effects of alien parasites on native hosts. Int. J. Parasitol. Parasites Wildl. 3, 171–177. https://doi.org/10.1016/j.ijppaw.2014.04.002

Ma, M., Huang, Y., Gong, Z., Zhuang, L., Li, C., Yang, H., Tong, Y., Liu, W., Cao, W., 2011. Discovery of DNA viruses in wild-caught mosquitoes using small RNA high throughput sequencing. PLoS One 6, 1–7. https://doi.org/10.1371/journal.pone.0024758

MacDonald, D.W., 1979. The Flexible Social System of the Golden Jackal , Canis aureus. Behav. Ecol. Sociobiol. 5, 17–38.

Mace, G.M., Norris, K., Fitter, A.H., 2012. Biodiversity and ecosystem services: A multilayered relationship. Trends Ecol. Evol. 27, 19–25. https://doi.org/10.1016/j.tree.2011.08.006

Magle, S.B., Samuel, M.D., Van Deelen, T.R., Robinson, S.J., Mathews, N.E., 2013. Evaluating Spatial Overlap and Relatedness of White-tailed Deer in a Chronic Wasting Disease Management Zone. PLoS One 8, e56568. https://doi.org/10.1371/journal.pone.0056568

Mamanova, L., Coffey, A.J., Scott, C.E., Kozarewa, I., Turner, E.H., Kumar, A., Howard, E., Shendure, J., Turner, D.J., 2010. Target-enrichment strategies for next-generation sequencing. Nat. Methods 7, 111–118. https://doi.org/10.1038/nmeth.1419

Mancini, M. V., Damiani, C., Accoti, A., Tallarita, M., Nunzi, E., Cappelli, A., Bozic, J., Catanzani, R., Rossi, P., Valzano, M., Serrao, A., Ricci, I., Spaccapelo, R., Favia, G., 2018. Estimating bacteria diversity in different organs of nine species of mosquito by next generation sequencing. BMC Microbiol. 18, 1–10. https://doi.org/10.1186/s12866-018-1266-9

Manel, S., Holderegger, R., 2013. Ten years of landscape genetics. Trends Ecol. Evol. 28, 614–621. https://doi.org/10.1016/j.tree.2013.05.012

Marcogliese, D.J., 2005. Parasites of the superorganism: Are they indicators of ecosystem health? Int. J. Parasitol. 35, 705–716. https://doi.org/10.1016/j.ijpara.2005.01.015

Martel, A., Spitzen-van der Sluijs, A., Blooi, M., Bert, W., Ducatelle, R., Fisher, M.C., Woeltjes, A., Bosman, W., Chiers, K., Bossuyt, F., Pasmans, F., 2013. Batrachochytrium salamandrivorans sp. nov. causes lethal chytridiomycosis in amphibians. Proc. Natl. Acad. Sci. 110, 15325–15329. https://doi.org/10.1073/pnas.1307356110

Martínez, J., Vásquez, R.A., Venegas, C., Merino, S., 2015. Molecular characterisation of haemoparasites in forest birds from Robinson Crusoe Island: Is the Austral Thrush a potential threat to endemic birds? Bird Conserv. Int. 25, 139–152. https://doi.org/10.1017/S0959270914000227

Martinsen, E.S., Perkins, S.L., Schall, J.J., 2008. A three-genome phylogeny of malaria parasites (Plasmodium and closely related genera): evolution of life-history traits and host switches. Mol. Phylogenet. Evol. 47, 261–73. https://doi.org/10.1016/j.ympev.2007.11.012

Marzal, A., Garc'ia-Longoria, L., Cárdenas Callirgos, J.M., Sehgal, R.N.M., 2015. Invasive avian malaria as an emerging parasitic disease in native birds of Peru. Biol. Invasions 17, 39–45. https://doi.org/10.1007/s10530-014-0718-x

McCormack, J.E., Hird, S.M., Zellmer, A.J., Carstens, B.C., Brumfield, R.T., 2013. Applications of next-generation sequencing to phylogeography and phylogenetics. Mol. Phylogenet. Evol. 66, 526–538. https://doi.org/10.1016/j.ympev.2011.12.007

Mccormack, J.E., Huang, H., Knowles, L.L., 2009. Sky islands, in: Gillespie, R., Clague, D. (Eds.), Encyclopedia of Islands. University of California Press, Berkeley , CA, pp. 839–843. https://doi.org/http://dx.doi.org/10.1302/0301-620X.79B4.7428

McGrew, A.K., O'Hara, T.M., Stricker, C.A., Salman, M.D., Van Bonn, W., Gulland, F.M.D., Whiting, A., Ballweber, L.R., 2018. Ecotoxicoparasitology of the gastrointestinal tracts of

pinnipeds: the effect of parasites on the potential bioavailability of total mercury (THg). Sci. Total Environ. 631–632, 233–238. https://doi.org/10.1016/j.scitotenv.2018.02.173

Medeiros, M.C.I., Hamer, G.L., Ricklefs, R.E., 2013. Host compatibility rather than vector-host-encounter rate determines the host range of avian Plasmodium parasites. Proc. Biol. Sci. 280, 20122947. https://doi.org/10.1098/rspb.2012.2947

Medeiros, M.C.I., Ricklefs, R.E., Brawn, J.D., Hamer, G.L., 2015. Plasmodium prevalence across avian host species is positively associated with exposure to mosquito vectors. Parasitology 142, 1612–1620. https://doi.org/10.1017/S0031182015001183

Medzhitov, R., Schneider, D.S., Soares, M.P., 2012. Disease tolerance as a defense strategy. Science 335, 936–41. https://doi.org/10.1126/science.1214935

Meher-Homji, V.M., 1967. Phytogeography of the South Indian Hill Stations. Bull. Torrey Bot. Club 94, 230–242. https://doi.org/10.2307/2483901

Meher-Homji, V.M., 1965. Ecological status of the montane grasslands of the south Indian hills: A phytogeographic reassessment. Indian For. 91, 210–215.

Mellor, P.S., Boorman, J., Baylis, M., 2000. Culicoides biting midges: their role as arbovirus vectors. Annu. Rev. Entomol. 45, 307–340. https://doi.org/10.1146/annurev.ento.45.1.307

Merino, S., Hennicke, J., Martínez, J., Ludynia, K., Torres, R., Work, T.M., Stroud, S., Masello, J.F., Quillfeldt, P., 2012. Infection by Haemoproteus parasites in four species of frigatebirds and the description of a new species of Haemoproteus (Haemosporida: Haemoproteidae). J. Parasitol. 98, 388–397. https://doi.org/10.1645/GE-2415.1

Merkle, D., Middendorf, M., Wieseke, N., 2010. A parameter-adaptive dynamic programming approach for inferring cophylogenies. BMC Bioinformatics. https://doi.org/10.1186/1471-

2105-11-S1-S60

Merrill, L., Levengood, J.M., England, J.C., Osborn, J.M., Hagy, H.M., 2018. Blood parasite infection linked to condition of spring-migrating Lesser Scaup (*Aythya affinis*). Can. J. Zool. 96, 1145–1152. https://doi.org/10.1139/cjz-2017-0307

Miaud, C., Arnal, V., Poulain, M., Valentini, A., Dejean, T., 2019. eDNA increases the detectability of ranavirus infection in an alpine amphibian population. Viruses 11, 1–15. https://doi.org/10.3390/v11060526

Miller, M.A., Pfeiffer, W., Schwartz, T., 2010. Creating the CIPRES Science Gateway for inference of large phylogenetic trees, in: 2010 Gateway Computing Environments Workshop, GCE 2010. https://doi.org/10.1109/GCE.2010.5676129

Mitchell, J., Vitikainen, E.I.K., Wells, D.A., Cant, M.A., Nichols, H.J., 2017. Heterozygosity but not inbreeding coefficient predicts parasite burdens in the banded mongoose. J. Zool. 302, 32–39. https://doi.org/10.1111/jzo.12424

Moens, M.A.J., Pérez-Tris, J., 2016. Discovering potential sources of emerging pathogens: South America is a reservoir of generalist avian blood parasites. Int. J. Parasitol. 46, 41–49. https://doi.org/10.1016/j.ijpara.2015.08.001

Moller, A.P., 1990. Parasites and sexual selection: Current status of the Hamilton and Zuk hypothesis. J. Evol. Biol. 3, 319–328. https://doi.org/10.1046/j.1420-9101.1990.3050319.x

Møller, A.P., 1992. Parasites differentially increase the degree of fluctuating asymmetry in secondary sexual characters. J. Evol. Biol. 5, 691–699. https://doi.org/10.1046/j.1420-9101.1992.5040691.x

Møller, A.P., Christe, P., Erritzøe, J., Mavarez, J., Moller, A.P., Erritzoe, J., 1998. Condition,

Disease and Immune Defence. Oikos 83, 301. https://doi.org/10.2307/3546841

Monello, R.J., Galloway, N.L., Powers, J.G., Madsen-Bouterse, S.A., Edwards, W.H., Wood, M.E., O'Rourke, K.I., Wild, M.A., 2017. Pathogen-mediated selection in free-ranging elk populations infected by chronic wasting disease. Proc. Natl. Acad. Sci. 201707807. https://doi.org/10.1073/pnas.1707807114

Mooers, A., Faith, D.P., Maddison, W.P., 2008. Converting endangered species categories to probabilities of extinction for phylogenetic conservation prioritization. PLoS One 3, 1–5. https://doi.org/10.1371/journal.pone.0003700

Morley, N.J., Lewis, J.W., 2006. Anthropogenic pressure on a molluscan-trematode community over a long-term period in the Basingstoke Canal, UK, and its implications for ecosystem health. Ecohealth 3, 269–280. https://doi.org/10.1007/s10393-006-0058-0

Morse, S.S., 1995. Factors in the emergence of infectious diseases. Emerg. Infect. Dis. 1, 7–15. https://doi.org/10.3201/eid0101.950102

Myers, N., Myers, N., Mittermeier, R. a, Mittermeier, R. a, Fonseca, Gustavo a B, Fonseca, G a B, Kent, J., Kent, J., 2000. Biodiversity hotspots for conservation priorities. Nature 403, 853–8. https://doi.org/10.1038/35002501

Nachev, M., Sures, B., 2016. Environmental parasitology: Parasites as accumulation bioindicators in the marine environment. J. Sea Res. 113, 45–50. https://doi.org/10.1016/j.seares.2015.06.005

Newhouse, D.J., Balakrishnan, C.N., 2015. High major histocompatibility complex class I polymorphism despite bottlenecks in wild and domesticated populations of the zebra finch (*Taeniopygia guttata*). BMC Evol. Biol. 15, 1–11. https://doi.org/10.1186/s12862-015-

0546-3

Newman, S.H., Hill, N.J., Spragens, K.A., Janies, D., Voronkin, I.O., Prosser, D.J., Yan, B., Lei, F., Batbayar, N., Natsagdorj, T., Bishop, C.M., Butler, P.J., Wikelski, M., Balachandran, S., Mundkur, T., Douglas, D.C., Takekawa, J.Y., 2012. Eco-virological approach for assessing the role of wild birds in the spread of avian influenza H5N1 along the central Asian flyway. PLoS One 7, 1–12. https://doi.org/10.1371/journal.pone.0030636

Niebuhr, C.N., Poulin, R., Tompkins, D.M., Bensch, S., Križanauskienė, A., Hasselquist, D., 2016. Is Avian Malaria Playing a Role in Native Bird Declines in New Zealand? Testing Hypotheses along an Elevational Gradient. PLoS One 11, e0165918. https://doi.org/10.1371/journal.pone.0165918

Nielsen, R., Bustamante, C., Clark, A.G., Glanowski, S., Sackton, T.B., Hubisz, M.J., Fledel-Alon, A., Tanenbaum, D.M., Civello, D., White, T.J., Sninsky, J.J., Adams, M.D., Cargill, M., 2005. A scan for positively selected genes in the genomes of humans and chimpanzees. PLoS Biol. 3, 0976–0985. https://doi.org/10.1371/journal.pbio.0030170

Nipperess, D.A., Matsen, F.A., 2013. The mean and variance of phylogenetic diversity under rarefaction. Methods Ecol. Evol. https://doi.org/10.1111/2041-210X.12042

Niskanen, A.K., Kennedy, L.J., Ruokonen, M., Kojola, I., Lohi, H., Isomursu, M., Jansson, E., Pyhäjärvi, T., Aspi, J., 2014. Balancing selection and heterozygote advantage in major histocompatibility complex loci of the bottlenecked Finnish wolf population. Mol. Ecol. 23, 875–889. https://doi.org/10.1111/mec.12647

Noble, W.A., 1967. The Shifting Balance of Grasslands, Shola Forests, and Planted Trees on the Upper Nilgiris, Southern India. Indian For. 93, 691–693.

Nunes, L.A., Turvey, S.T., Rosindell, J., 2015. The price of conserving avian phylogenetic diversity: A global prioritization approach. Philos. Trans. R. Soc. B Biol. Sci. 370, 1–11. https://doi.org/10.1098/rstb.2014.0004

O'Hanlon, S.J., Rieux, A., Farrer, R.A., Rosa, G.M., Waldman, B., Bataille, A., Kosch, T.A., Murray, K.A., Brankovics, B., Fumagalli, M., Martin, M.D., Wales, N., Alvarado-Rybak, M., Bates, K.A., Berger, L., Böll, S., Brookes, L., Clare, F., Courtois, E.A., Cunningham, A.A., Doherty-Bone, T.M., Ghosh, P., Gower, D.J., Hintz, W.E., Höglund, J., Jenkinson, T.S., Lin, C.-F., Laurila, A., Loyau, A., Martel, A., Meurling, S., Miaud, C., Minting, P., Pasmans, F., Schmeller, D.S., Schmidt, B.R., Shelton, J.M.G., Skerratt, L.F., Smith, F., Soto-Azat, C., Spagnoletti, M., Tessa, G., Toledo, L.F., Valenzuela-Sánchez, A., Verster, R., Vörös, J., Webb, R.J., Wierzbicki, C., Wombwell, E., Zamudio, K.R., Aanensen, D.M., James, T.Y., Gilbert, M.T.P., Weldon, C., Bosch, J., Balloux, F., Garner, T.W.J., Fisher, M.C., 2018. Recent Asian origin of chytrid fungi causing global amphibian declines. Science 360, 621–627. https://doi.org/10.1126/science.aar1965

Oksanen, J., Blanchet, F.G., Kindt, R., Legendre, P., Minchin, P.R., O'Hara, R.B., Simpson, G.L., Solymos, P., Henry, M., Stevens, H., Wagner, H., 2016. Vegan: community ecology package. R Packag. version 2.4-1. https//CRAN.R-project.org/package=vegan.

Olsson-Pons, S., Clark, N.J., Ishtiaq, F., Clegg, S.M., 2015. Differences in host species relationships and biogeographic influences produce contrasting patterns of prevalence, community composition and genetic structure in two genera of avian malaria parasites in southern Melanesia. J. Anim. Ecol. 84, 985–998. https://doi.org/10.1111/1365-2656.12354

Ortego, J., Aparicio, J.M., Calabuig, G., Cordero, P.J., 2007a. Risk of ectoparasitism and genetic

diversity in a wild lesser kestrel population. Mol. Ecol. 16, 3712–3720. https://doi.org/10.1111/j.1365-294X.2007.03406.x

Ortego, J., Cordero, P.J., Aparicio, J.M., Calabuig, G., 2007b. No relationship between individual genetic diversity and prevalence of avian malaria in a migratory kestrel. Mol. Ecol. 16, 4858–4866. https://doi.org/10.1111/j.1365-294X.2007.03554.x

Ortiz-Catedral, L., Brunton, D., Stidworthy, M.F., Elsheikha, H.M., Pennycott, T., Schulze, C., Braun, M., Wink, M., Gerlach, H., Pendl, H., Gruber, A.D., Ewen, J., Pérez-Tris, J., Valkiūnas, G., Olias, P., 2019. Haemoproteus minutus is highly virulent for Australasian and South American parrots. Parasit. Vectors 12, 40. https://doi.org/10.1186/s13071-018-3255-0

Ostfeld, R.S., 2013. A Candide response to Panglossian accusations by Randolph and Dobson: biodiversity buffers disease. Parasitology 140, 1196–1198. https://doi.org/10.1017/S0031182013000541

Ostfeld, R.S., Keesing, F., 2012. Effects of Host Diversity on Infectious Disease. Annu. Rev. Ecol. Evol. Syst. 43, 157–184. https://doi.org/10.1146/annurev-ecolsys-102710-145022

Otto, T.D., Böhme, U., Jackson, A.P., Hunt, M., Franke-Fayard, B., Hoeijmakers, W.A.M., Religa, A.A., Robertson, L., Sanders, M., Ogun, S.A., Cunningham, D., Erhart, A., Billker, O., Khan, S.M., Stunnenberg, H.G., Langhorne, J., Holder, A.A., Waters, A.P., Newbold, C.I., Pain, A., Berriman, M., Janse, C.J., 2014a. A comprehensive evaluation of rodent malaria parasite genomes and gene expression. BMC Biol. 12, 86. https://doi.org/10.1186/s12915-014-0086-0

Otto, T.D., Rayner, J.C., Böhme, U., Pain, A., Spottiswoode, N., Sanders, M., Quail, M.,

Ollomo, B., Renaud, F., Thomas, A.W., Prugnolle, F., Conway, D.J., Newbold, C., Berriman, M., 2014b. Genome sequencing of chimpanzee malaria parasites reveals possible pathways of adaptation to human hosts. Nat. Commun. 5, 4754. https://doi.org/10.1038/ncomms5754

Pafčo, B., Čížková, D., Kreisinger, J., Hasegawa, H., Vallo, P., Shutt, K., Todd, A., Petrželková, K.J., Modrý, D., 2018. Metabarcoding analysis of strongylid nematode diversity in two sympatric primate species. Sci. Rep. 8, 1–11. https://doi.org/10.1038/s41598-018-24126-3

Pagán, I., García-Arenal, F., 2018. Tolerance to plant pathogens: Theory and experimental evidence. Int. J. Mol. Sci. 19, 810. https://doi.org/10.3390/ijms19030810

Pagel, M., 1999. Inferring the historical patterns of biological evolution. Nature 401, 877–884. https://doi.org/10.1038/44766

Pain, A., Böhme, U., Berry, A.E., Mungall, K., Finn, R.D., Jackson, A.P., Mourier, T., Mistry, J., Pasini, E.M., Aslett, M.A., Balasubrammaniam, S., Borgwardt, K., Brooks, K., Carret, C., Carver, T.J., Cherevach, I., Chillingworth, T., Clark, T.G., Galinski, M.R., Hall, N., Harper, D., Harris, D., Hauser, H., Ivens, A., Janssen, C.S., Keane, T., Larke, N., Lapp, S., Marti, M., Moule, S., Meyer, I.M., Ormond, D., Peters, N., Sanders, M., Sanders, S., Sargeant, T.J., Simmonds, M., Smith, F., Squares, R., Thurston, S., Tivey, A.R., Walker, D., White, B., Zuiderwijk, E., Churcher, C., Quail, M.A., Cowman, A.F., Turner, C.M.R., Rajandream, M.A., Kocken, C.H.M., Thomas, A.W., Newbold, C.I., Barrell, B.G., Berriman, M., 2008. The genome of the simian and human malaria parasite Plasmodium knowlesi. Nature 455, 799–803. https://doi.org/10.1038/nature07306

Palinauskas, V., Kriz, A., Iezhova, T.A., Bolshakov, C. V, Jönsson, J., 2013. A new method for

isolation of purified genomic DNA from haemosporidian parasites inhabiting nucleated red blood cells. Exp. Parasitol. 133, 275–280. https://doi.org/10.1016/j.exppara.2012.12.003

Palinauskas, V., Valkiūnas, G., Bolshakov, C. V, Bensch, S., 2008. Plasmodium relictum (lineage P-SGS1): effects on experimentally infected passerine birds. Exp. Parasitol. 120, 372–80. https://doi.org/10.1016/j.exppara.2008.09.001

Palinauskas, V., Ziegyte, R., Iezhova, T.A., Bernotiene, R., Valki, G., 2016. Description, molecular characterisation, diagnostics and life cycle of Plasmodium elongatum (lineage pERIRUB01), the virulent avian malaria parasite. Int. J. Parasitol. 46, 697–707. https://doi.org/10.1016/j.ijpara.2016.05.005

Panzera, Y., Sarute, N., Iraola, G., Hernández, M., Pérez, R., 2015. Molecular phylogeography of canine distemper virus: Geographic origin and global spreading. Mol. Phylogenet. Evol. 92, 147–154. https://doi.org/10.1016/j.ympev.2015.06.015

Paradis, E., Schliep, K., 2019. Ape 5.0: An environment for modern phylogenetics and evolutionary analyses in R. Bioinformatics 35, 526–528. https://doi.org/10.1093/bioinformatics/bty633

Parham, P.E., Waldock, J., Christophides, G.K., Hemming, D., Agusto, F., Evans, K.J., Fefferman, N.H., Gaff, H., Gumel, A., LaDeau, S., Lenhart, S., Mickens, R.E., Naumova, E.N., Ostfeld, R.S., Ready, P.D., Thomas, M.B., Velasco-Hernandez, J., Michael, E., 2015. Climate, environmental and socio-economic change: weighing up the balance in vector-borne disease transmission. Philos. Trans. R. Soc. B 370, 20130551. https://doi.org/10.1098/rstb.2013.0551

Parker, B.J., Garcia, J.R., Gerardo, N.M., 2014. Genetic variation in resistance and fecundity

tolerance in a natural host-pathogen interaction. Evolution 68, 2421–2429. https://doi.org/10.1111/evo.12418

Parker, I.M., Saunders, M., Bontrager, M., Weitz, A.P., Hendricks, R., Magarey, R., Suiter, K., Gilbert, G.S., 2015. Phylogenetic structure and host abundance drive disease pressure in communities. Nature 520, 542–544. https://doi.org/10.1038/nature14372

Patz, J.A., Graczyk, T.K., Geller, N., Vittor, A.Y., 2000. Effects of environmental change on emerging parasitic diseases. Int. J. Parasitol. 30, 1395–1405.

Pauli, M., Chakarov, N., Rupp, O., Kalinowski, J., Goesmann, A., Sorenson, M.D., Krüger, O., Hoffman, J.I., 2015. De novo assembly of the dual transcriptomes of a polymorphic raptor species and its malarial parasite. BMC Genomics 16, 1038. https://doi.org/10.1186/s12864-015-2254-1

Peh, K.S..-H.., 2007. Potential Effects of Climate Change on Elevational Distributions of Tropical Birds in Southeast Asia. Condor 109, 437–441.

Peig, J., Green, A.J., 2009. New perspectives for estimating body condition from mass/length data: the scaled mass index as an alternative method. Oikos 118, 1883–1891. https://doi.org/10.1111/j.1600-0706.2009.17643.x

Pérez-del Olmo, A., Raga, J.A., Kostadinova, A., Fernández, M., 2007. Parasite communities in Boops boops (L.) (Sparidae) after the Prestige oil-spill: Detectable alterations. Mar. Pollut. Bull. 54, 266–276. https://doi.org/10.1016/j.marpolbul.2006.10.003

Pérez-Rodríguez, A., de la Hera, I., Fernández-González, S., Pérez-Tris, J., 2014. Global warming will reshuffle the areas of high prevalence and richness of three genera of avian blood parasites. Glob. Chang. Biol. 20, 2406–2416. https://doi.org/10.1111/gcb.12542

Pérez-Rodríguez, A., Fernández-González, S., de la Hera, I., Pérez-Tris, J., 2013. Finding the appropriate variables to model the distribution of vector-borne parasites with different environmental preferences: climate is not enough. Glob. Chang. Biol. 19, 3245–53. https://doi.org/10.1111/gcb.12226

Perkins, S.L., 2014a. Malaria's many mates: past, present, and future of the systematics of the order Haemosporida. J. Parasitol. 100, 11–25. https://doi.org/10.1645/13-362.1

Perkins, S.L., 2014b. Malaria's Many Mates: Past, Present, and Future of the Systematics of the Order Haemosporida. J. Parasitol. 100, 11–25. https://doi.org/10.1645/13-362.1

Perkins, S.L., Schall, J.J., 2002. A molecular phylogeny of malarial parasites recovered from cytochrome b gene sequences. J. Parasitol. 88, 972–978. https://doi.org/10.1645/0022-3395(2002)088[0972:AMPOMP]2.0.CO;2

Petren, K., 2013. The evolution of landscape genetics. Evolution 67, 3383–3385. https://doi.org/10.1111/evo.12278

Pinheiro, L.B., Coleman, V.A., Hindson, C.M., Herrmann, J., Hindson, B.J., Bhat, S., Emslie, K.R., 2012. Evaluation of a droplet digital polymerase chain reaction format for DNA copy number quantification. Anal. Chem. 84, 1003–1011. https://doi.org/10.1021/ac202578x

Plummer, M., Best, N., Cowles, K., Vines, K., 2006. CODA: Convergence Diagnosis and Output Analysis for MCMC. R News 6, 7–11.

Poorten, T.J., Rosenblum, E.B., 2016. Comparative study of host response to chytridiomycosis in a susceptible and a resistant toad species. Mol. Ecol. 25, 5663–5679. https://doi.org/10.1111/mec.13871

Posada, D., 2008. jModelTest: Phylogenetic model averaging. Mol. Biol. Evol. 25, 1253–1256.

https://doi.org/10.1093/molbev/msn083

Poulin, R., Krasnov, B.R., Mouillot, D., 2011. Host specificity in phylogenetic and geographic space. Trends Parasitol. 27, 355–361. https://doi.org/10.1016/j.pt.2011.05.003

Poulin, R., Morand, S., 2000. The Diversity of Parasites. Q. Rev. Biol. 75, 277–293. https://doi.org/10.1086/393500

Pounds, J.A., Bustamante, M.R., Coloma, L.A., Consuegra, J.A., Fogden, M.P.L., Foster, P.N., La Marca, E., Masters, K.L., Merino-Viteri, A., Puschendorf, R., Ron, S.R., Sánchez-Azofeifa, G.A., Still, C.J., Young, B.E., 2006. Widespread amphibian extinctions from epidemic disease driven by global warming. Nature 439, 161–167. https://doi.org/10.1038/nature04246

Prasad, V., Farooqui, A., Tripathi, S.K.M., Garg, R., Thakur, B., 2009. Evidence of late Palaeocene-early eocene equatorial rain forest refugia in southern Western Ghats, India. J. Biosci. 34, 777–797. https://doi.org/10.1007/s12038-009-0062-y

Praveen Karanth, K., 2015. An island called India: Phylogenetic patterns across multiple taxonomic groups reveal endemic radiations. Curr. Sci. 108, 1847–1851.

Prenter, J., MacNeil, C., Dick, J.T.A., Dunn, A.M., 2004. Roles of parasites in animal invasions. Trends Ecol. Evol. 19, 385–390. https://doi.org/10.1016/j.tree.2004.05.002

Preston, D.L., Jacobs, A.Z., Orlofske, S.A., Johnson, P.T.J., 2014. Complex life cycles in a pond food web: Effects of life stage structure and parasites on network properties, trophic positions and the fit of a probabilistic niche model. Oecologia 174, 953–965. https://doi.org/10.1007/s00442-013-2806-5

Preston, D.L., Mischler, J.A., Townsend, A.R., Johnson, P.T.J., 2016. Disease Ecology Meets

Ecosystem Science. Ecosystems 19, 737–748. https://doi.org/10.1007/s10021-016-9965-2

Preston, D.L., Orlofske, S.A., Lambden, J.P., Johnson, P.T.J., 2013. Biomass and productivity of trematode parasites in pond ecosystems. J. Anim. Ecol. 82, 509–517. https://doi.org/10.1111/1365-2656.12030

Price, P.W., Westoby, M., Rice, B., Atsatt, P.R., Fritz, R.S., Thompson, J.N., Mobley, K., 1986. Parasite Mediation in Ecological Interactions. Annu. Rev. Ecol. Syst. 17, 487–505. https://doi.org/10.1146/annurev.es.17.110186.002415

Prüter, H., Franz, M., Auls, S., Czirják, G., Greben, O., Greenwood, A.D., Lisitsyna, O., Syrota, Y., Sitko, J., Krone, O., 2018. Chronic lead intoxication decreases intestinal helminth species richness and infection intensity in mallards (Anas platyrhynchos). Sci. Total Environ. 644, 151–160. https://doi.org/10.1016/j.scitotenv.2018.06.297

R Core Team, 2019. R: A language and environment for statistical computing. R Found. Stat. Comput. Vienna, Austria. URL http//www.R-project.org/.

Råberg, L., Graham, A.L., Read, A.F., 2009. Decomposing health: tolerance and resistance to parasites in animals. Philos. Trans. R. Soc. Lond. B. Biol. Sci. 364, 37–49. https://doi.org/10.1098/rstb.2008.0184

Råberg, L., Sim, D., Read, A.F., 2007. Disentangling genetic variation for resistance and tolerance to infectious diseases in animals. Science 318, 812–4. https://doi.org/10.1126/science.1148526

Rambaut, A., 2012. FigTree v1. 4.0. A graphical viewer of phylogenetic trees. Inst. Evol. Biol. Univ. Edinburgh.

Ramírez, J.D., Herrera, G., Hernández, C., Cruz-Saavedra, L., Muñoz, M., Flórez, C., Butcher,

R., 2018. Evaluation of the analytical and diagnostic performance of a digital droplet polymerase chain reaction (ddPCR) assay to detect Trypanosoma cruzi DNA in blood samples. PLoS Negl. Trop. Dis. 12, e0007063. https://doi.org/10.1371/journal.pntd.0007063

Randolph, S.E., Dobson, A.D.M., 2012. Pangloss revisited: a critique of the dilution effect and the biodiversity-buffers-disease paradigm. Parasitology 139, 847–863. https://doi.org/10.1017/S0031182012000200

Ranganathan, C.R., 1938. Studies in the Ecology of the Shola Grassland Vegetation of the Nilgiri Plateau.

Rasmussen, P.C., Anderton, J.C., 2005. Birds of South Asia: the Ripley Guide. Volume 2: attributes and status. Smithsonian Institution and Lynx Edicions, Washington D.C. and Barcelona.

Rebollar, E.A., Antwis, R.E., Becker, M.H., Belden, L.K., Bletz, M.C., Brucker, R.M., Harrison, X.A., Hughey, M.C., Kueneman, J.G., Loudon, A.H., McKenzie, V., Medina, D., Minbiole, K.P.C., Rollins-Smith, L.A., Walke, J.B., Weiss, S., Woodhams, D.C., Harris, R.N., 2016. Using “omics” and integrated multi-omics approaches to guide probiotic selection to mitigate chytridiomycosis and other emerging infectious diseases. Front. Microbiol. 7, 68. https://doi.org/10.3389/fmicb.2016.00068

Reuter, S., Connor, T.R., Barquist, L., Walker, D., Feltwell, T., Harris, S.R., Fookes, M., Hall, M.E., Petty, N.K., Fuchs, T.M., Corander, J., Dufour, M., Ringwood, T., Savin, C., Bouchier, C., Martin, L., Miettinen, M., Shubin, M., Riehm, J.M., Laukkanen-Ninios, R., Sihvonen, L.M., Siitonen, A., Skurnik, M., Falcao, J.P., Fukushima, H., Scholz, H.C., Prentice, M.B., Wren, B.W., Parkhill, J., Carniel, E., Achtman, M., McNally, A., Thomson,

N.R., 2014. Parallel independent evolution of pathogenicity within the genus Yersinia. Proc. Natl. Acad. Sci. 111, 6768–6773. https://doi.org/10.1073/pnas.1317161111

Richard, F.A., Sehgal, R.N.M., Jones, H.I., Smith, T.B., Matbouli, E.L., 2002. A Comparative Analysis of PCR-Based Detection Methods for Avian Malaria 88, 819–822.

Ricklefs, R.E., Fallon, S.M., Bermingham, E., 2004. Evolutionary relationships, cospeciation, and host switching in avian malaria parasites. Syst. Biol. 53, 111–119. https://doi.org/10.1080/10635150490264987

Ricklefs, R.E., Outlaw, D.C., Svensson-coelho, M., Medeiros, M.C.I., Ellis, V.A., 2014. Species formation by host shifting in avian malaria parasites. Proc. Natl. Acad. Sci. 111, 14816–14821. https://doi.org/10.1073/pnas.1416356111

Ricklefs, R.E., Swanson, B.L., Fallon, S.M., Martínez-Abraín, A., Scheuerlein, A., Gray, J., Latta, S.C., 2005. Community relationships of avian malaria parasites. Ecol. Monogr. 75, 543–559.

Rijks, J.M., Hoffman, J.I., Kuiken, T., Osterhaus, A.D.M.E., Amos, W., 2008. Heterozygosity and lungworm burden in harbour seals (Phoca vitulina). Heredity 100, 587–593. https://doi.org/10.1038/hdy.2008.18

Riper, C. van, Riper, S.G. Van, Goff, M.L., Laird, M., 1986. The Epizootiology and Ecological Significance of Malaria in Hawaiian Land Birds. Ecol. Monogr. 56, 327–344.

Rivero, A., Gandon, S., 2018. Evolutionary Ecology of Avian Malaria: Past to Present. Trends Parasitol. 34, 712–726. https://doi.org/10.1016/j.pt.2018.06.002

Robar, N., Burness, G., Murray, D.L., 2010. Tropics, trophics and taxonomy: The determinants of parasite-associated host mortality. Oikos 119, 1273–1280. https://doi.org/10.1111/j.1600-

0706.2009.18292.x

Robin, V.V., Gupta, P., Thatte, P., Ramakrishnan, U., 2015a. Islands within islands: two montane palaeo-endemic birds impacted by recent anthropogenic fragmentation. Mol. Ecol. 24, 3572–3584. https://doi.org/10.1111/mec.13266

Robin, V.V., Nandini, R., 2012. Shola habitats on sky islands: status of research on montane forests and grasslands in southern India. Curr. Sci. 103.

Robin, V.V., Sinha, A., Ramakrishnan, U., 2010. Ancient Geographical Gaps and Paleo-Climate Shape the Phylogeography of an Endemic Bird in the Sky Islands of Southern India. PLoS One 5. https://doi.org/10.1371/journal.pone.0013321

Robin, V.V., Vishnudas, C.K., Gupta, P., Ramakrishnan, U., 2015b. Deep and wide valleys drive nested phylogeographic patterns across a montane bird community. Proc. R. Soc. B Biol. Sci. 282, 20150861. https://doi.org/10.1098/rspb.2015.0861

Robin, V.V., Vishnudas, C.K., Gupta, P., Rheindt, F.E., Hooper, D.M., Ramakrishnan, U., Reddy, S., 2017. Two new genera of songbirds represent endemic radiations from the Shola Sky Islands of the Western Ghats, India. BMC Evol. Biol. 17, 31. https://doi.org/10.1186/s12862-017-0882-6

Robin, V.V., Vishnudas, C.K., Ramakrishnan, U., 2014. Reassessment of the distribution and threat status of the Western Ghats endemic bird, Nilgiri Pipit Anthus nilghiriensis. Curr. Sci. 107, 622–630.

Rodríguez-Ruano, S.M., Škochová, V., Rego, R.O.M., Schmidt, J.O., Roachell, W., Hypša, V., Nováková, E., 2018. Microbiomes of North American triatominae: The grounds for Chagas disease epidemiology. Front. Microbiol. 9, 1–11. https://doi.org/10.3389/fmicb.2018.01167

Rogalski, M.A., Gowler, C.D., Shaw, C.L., Hufbauer, R.A., Duffy, M.A., 2017. Human drivers of ecological and evolutionary dynamics in emerging and disappearing infectious disease systems. Philos. Trans. R. Soc. B Biol. Sci. 372, 20160043. https://doi.org/10.1098/rstb.2016.0043

Ronquist, F., Huelsenbeck, J.P., 2003. MrBayes 3: Bayesian phylogenetic inference under mixed models. Bioinformatics 19, 1572–1574. https://doi.org/10.1093/bioinformatics/btg180

Rosenblum, E.B., James, T.Y., Zamudio, K.R., Poorten, T.J., Ilut, D., Rodriguez, D., Eastman, J.M., Richards-Hrdlicka, K., Joneson, S., Jenkinson, T.S., Longcore, J.E., Parra Olea, G., Toledo, L.F., Arellano, M.L., Medina, E.M., Restrepo, S., Flechas, S.V., Berger, L., Briggs, C.J., Stajich, J.E., 2013. Complex history of the amphibian-killing chytrid fungus revealed with genome resequencing data. Proc. Natl. Acad. Sci. 110, 9385–9390. https://doi.org/10.1073/pnas.1300130110

Ruess, R.W., McFarland, J.M., Trummer, L.M., Rohrs-Richey, J.K., 2009. Disease-mediated declines in N-fixation inputs by Alnus tenuifolia to early-successional floodplains in interior and south-central Alaska. Ecosystems 12, 489–502. https://doi.org/10.1007/s10021-009-9237-5

Ruiz-López, M.J., Monello, R.J., Gompper, M.E., Eggert, L.S., 2012. The Effect and Relative Importance of Neutral Genetic Diversity for Predicting Parasitism Varies across Parasite Taxa. PLoS One 7, e45404. https://doi.org/10.1371/journal.pone.0045404

Rushton, S.P., Lurz, P.W.W., Gurnell, J., Fuller, R., Society, B.E., Ecology, A., 2000. Modelling the Spatial Dynamics of Parapoxvirus Disease in Red and Grey Squirrels : A Possible Cause of the Decline in the Red Squirrel in the UK ? J. Appl. Ecol. 37, 997–1012.

https://doi.org/10.1046/j.1365-2664.2000.00553.x

Rutledge, G.G., Böhme, U., Sanders, M., Reid, A.J., Cotton, J.A., Maiga-Ascofare, O., Djimdé, A.A., Apinjoh, T.O., Amenga-Etego, L., Manske, M., Barnwell, J.W., Renaud, F., Ollomo, B., Prugnolle, F., Anstey, N.M., Auburn, S., Price, R.N., McCarthy, J.S., Kwiatkowski, D.P., Newbold, C.I., Berriman, M., Otto, T.D., 2017. Plasmodium malariae and P. ovale genomes provide insights into malaria parasite evolution. Nature 542, 101–104. https://doi.org/10.1038/nature21038

Rzanny, M., Voigt, W., 2012. Complexity of multitrophic interactions in a grassland ecosystem depends on plant species diversity. J. Anim. Ecol. https://doi.org/10.1111/j.1365-2656.2012.01951.x

Sakkas, H., Economou, V., Papadopoulou, C., 2016. Zika virus infection: Past and present of another emerging vector-borne disease. J. Vector Borne Dis.

Sánchez, C.A., Becker, D.J., Teitelbaum, C.S., Barriga, P., Brown, L.M., Majewska, A.A., Hall, R.J., Altizer, S., 2018. On the relationship between body condition and parasite infection in wildlife: a review and meta-analysis. Ecol. Lett. 21, 1869–1884. https://doi.org/10.1111/ele.13160

Santiago-Alarcon, D., Palinauskas, V., Schaefer, H.M., 2012. Diptera vectors of avian Haemosporidian parasites: untangling parasite life cycles and their taxonomy. Biol. Rev. Camb. Philos. Soc. 87, 928–64. https://doi.org/10.1111/j.1469-185X.2012.00234.x

Sato, C.F., Lindenmayer, D.B., 2018. Meeting the global challenge. Conserv. Lett. 11, 1–7. https://doi.org/10.1111/conl.12348

Sato, T., Watanabe, K., Kanaiwa, M., Niizuma, Y., Harada, Y., Lafferty, K.D., 2011.

Nematomorph parasites drive energy flow through a riparian ecosystem. Ecology 92, 201–207. https://doi.org/10.1890/09-1565.1

Savage, A.E., Zamudio, K.R., 2016. Adaptive tolerance to a pathogenic fungus drives major histocompatibility complex evolution in natural amphibian populations. Proc. R. Soc. B Biol. Sci. 283, 20153115. https://doi.org/10.1098/rspb.2015.3115

Scheuerlein, A., Ricklefs, R.E., 2004. Prevalence of blood parasites in European passeriform birds. Proc. R. Soc. London Ser. B Biol. Sci. 271, 1363–1370. https://doi.org/10.1098/rspb.2004.2726

Schneider, D.S., Ayres, J.S., 2008. Two ways to survive infection: what resistance and tolerance can teach us about treating infectious diseases. Nat. Rev. Immunol. 8, 889–895.

Schneider, J., Valentini, A., Dejean, T., Montarsi, F., Taberlet, P., Glaizot, O., Fumagalli, L., 2016. Detection of Invasive Mosquito Vectors Using Environmental DNA (eDNA) from Water Samples. PLoS One 11, e0162493. https://doi.org/10.1371/journal.pone.0162493

Schnell, I.B., Sollmann, R., Calvignac-Spencer, S., Siddall, M.E., Yu, D.W., Wilting, A., Gilbert, M.T.P., 2015. iDNA from terrestrial haematophagous leeches as a wildlife surveying and monitoring tool - prospects, pitfalls and avenues to be developed. Front. Zool. 12, 1–14. https://doi.org/10.1186/s12983-015-0115-z

Schoener, E.R., Banda, M., Howe, L., Castro, I.C., Alley, M.R., 2014. Avian malaria in New Zealand. N. Z. Vet. J. 62, 189–198. https://doi.org/10.1080/00480169.2013.871195

Schrader, M.S., Walters, E.L., James, F.C., Greiner, E.C., 2003. Seasonal Prevalence of a Haematozoan Parasite of Red-Bellied Woodpeckers (Melanerpes Carolinus) and its Association With Host Condition and Overwinter Survival. Auk 120, 130–137.

https://doi.org/10.1093/auk/120.1.130

Schradin, C., Lindholm, A.K., Johannesen, J., Schoepf, I., Yuen, C.H., König, B., Pillay, N., 2012. Social flexibility and social evolution in mammals: A case study of the African striped mouse (*Rhabdomys pumilio*). Mol. Ecol. 21, 541–553. https://doi.org/10.1111/j.1365-294X.2011.05256.x

Schrag, S., Wiener, P., 1995. Emerging infectious disease: what are the relative roles of ecology and evolution? TREE 0–5.

Schwabl, P., Llewellyn, M., Landguth, E.L., Andersson, B., Kitron, U., Costales, J.A., Ocaña, S., Grijalva, M.J., 2017. Prediction and prevention of parasitic diseases using a landscape genomics framework. Trends Parasitol. 33, 264–275. https://doi.org/10.1186/s40945-017-0033-9.Using

Schwensow, N., Mazzoni, C.J., Marmesat, E., Fickel, J., Peacock, D., Kovaliski, J., Sinclair, R., Cassey, P., Cooke, B., Sommer, S., 2017. High adaptive variability and virus-driven selection on major histocompatibility complex (MHC) genes in invasive wild rabbits in Australia. Biol. Invasions 19, 1255–1271. https://doi.org/10.1007/s10530-016-1329-5

Scolari, F., Casiraghi, M., Bonizzoni, M., 2019. Aedes spp. and Their Microbiota: A Review. Front. Microbiol. 10, 2036. https://doi.org/10.3389/fmicb.2019.02036

Seimon, T.A., Seimon, A., Daszak, P., Halloy, S.R.P., Schloegel, L.M., Aguilar, C.A., Sowell, P., Hyatt, A.D., Konecky, B., Simmons, J.E., 2007. Upward range extension of Andean anurans and chytridiomycosis to extreme elevations in response to tropical deglaciation. Glob. Chang. Biol. 13, 288–299. https://doi.org/10.1111/j.1365-2486.2006.01278.x

Selbach, C., Jorge, F., Dowle, E., Bennett, J., Chai, X., Doherty, J.-F., Eriksson, A., Filion, A.,

Hay, E., Herbison, R., Lindner, J., Park, E., Presswell, B., Ruehle, B., Sobrinho, P.M., Wainwright, E., Poulin, R., 2019. Parasitological research in the molecular age. Parasitology 146, 1361–1370. https://doi.org/10.1017/S0031182019000726

Sengupta, M.E., Hellström, M., Kariuki, H.C., Olsen, A., Thomsen, P.F., Mejer, H., Willerslev, E., Mwanje, M.T., Madsen, H., Kristensen, T.K., Stensgaard, A.S., Vennervald, B.J., 2019. Environmental DNA for improved detection and environmental surveillance of schistosomiasis. Proc. Natl. Acad. Sci. 116, 8931–8940. https://doi.org/10.1073/pnas.1815046116

Sepil, I., Lachish, S., Hinks, A.E., Sheldon, B.C., 2013. Mhc supertypes confer both qualitative and quantitative resistance to avian malaria infections in a wild bird population.

Seppälä, O., Jokela, J., 2008. Host manipulation as a parasite transmission strategy when manipulation is exploited by non-host predators. Biol. Lett. 4, 663–666. https://doi.org/10.1098/rsbl.2008.0335

Shultz, A.J., Sackton, T.B., 2019. Immune genes are hotspots of shared positive selection across birds and mammals. Elife 8, e41815. https://doi.org/10.7554/eLife.41815

Simpson, J.T., Wong, K., Jackman, S.D., Schein, J.E., Jones, S.J.M., Birol, I., 2009. ABySS: A parallel assembler for short read sequence data. Genome Res. 19, 1117–1123. https://doi.org/10.1101/gr.089532.108

Sin, Y.W., Annavi, G., Dugdale, H.L., Newman, C., Burke, T., MacDonald, D.W., 2014. Pathogen burden, co-infection and major histocompatibility complex variability in the European badger (*Meles meles*). Mol. Ecol. 23, 5072–5088. https://doi.org/10.1111/mec.12917

Skerratt, L.F., Berger, L., Speare, R., Cashins, S., McDonald, K.R., Phillott, A.D., Hines, H.B., Kenyon, N., 2007. Spread of chytridiomycosis has caused the rapid global decline and extinction of frogs. Ecohealth 4, 125–134. https://doi.org/10.1007/s10393-007-0093-5

Slatyer, R.A., Hirst, M., Sexton, J.P., 2013. Niche breadth predicts geographical range size: A general ecological pattern. Ecol. Lett. 16, 1104–1114. https://doi.org/10.1111/ele.12140

Sloan, S., Jenkins, C.N., Joppa, L.N., Gaveau, D.L.A., Laurance, W.F., 2014. Remaining natural vegetation in the global biodiversity hotspots. Biol. Conserv. 177, 12–24. https://doi.org/10.1016/j.biocon.2014.05.027

Smallbone, W., van Oosterhout, C., Cable, J., 2016. The effects of inbreeding on disease susceptibility: Gyrodactylus turnbulli infection of guppies, Poecilia reticulata. Exp. Parasitol. 167, 32–37. https://doi.org/10.1016/j.exppara.2016.04.018

Smith, K.F., Acevedo-Whitehouse, K., Pedersen, A.B., 2009. The role of infectious diseases in biological conservation. Anim. Conserv. 12, 1–12. https://doi.org/10.1111/j.1469-1795.2008.00228.x

Soares, M.P., Teixeira, L., Moita, L.F., 2017. Disease tolerance and immunity in host protection against infection. Nat. Rev. Immunol. 17, 83–96. https://doi.org/10.1038/nri.2016.136

Soman, K., Thara, K.G., Arakelyants, M.M., Golubyev, V.N., 1990. Mineral ages of pegmatites from the Palghat Gap region in Kerala and their tectonic significance. J. - Geol. Soc. India.

Sorensen, A., van Beest, F.M., Brook, R.K., 2013. Impacts of wildlife baiting and supplemental feeding on infectious disease transmission risk: A synthesis of knowledge. Prev. Vet. Med. 11, 1–8. https://doi.org/10.1016/j.prevetmed.2013.11.010

Soulé, M.E., 1985. What is conservation biology? Bioscience 35, 727–734.

Stadler, T., Bonhoeffer, S., 2013. Uncovering epidemiological dynamics in heterogeneous host populations using phylogenetic methods. Philos. Trans. R. Soc. B Biol. Sci. 368, 20120198. https://doi.org/10.1098/rstb.2012.0198

Stamatakis, A., 2014. RAxML version 8: A tool for phylogenetic analysis and post-analysis of large phylogenies. Bioinformatics. https://doi.org/10.1093/bioinformatics/btu033

Stevenson, B., Goltz, J., Massé, A., 2016. Preparing for and responding to recent incursions of racoon rabies variant into Canada. Canada Commun. Dis. Rep. 42, 125–129. https://doi.org/10.14745/ccdr.v42i06a03

Stucki, D., Ballif, M., Bodmer, T., Coscolla, M., Maurer, A.M., Droz, S., Butz, C., Borrell, S., Längle, C., Feldmann, J., Furrer, H., Mordasini, C., Helbling, P., Rieder, H.L., Egger, M., Gagneux, S., Fenner, L., 2015. Tracking a tuberculosis outbreak over 21 years: Strain-specific single-nucleotide polymorphism typing combined with targeted whole-genome sequencing, in: Journal of Infectious Diseases. Oxford University Press, pp. 1306–1316. https://doi.org/10.1093/infdis/jiu601

Sukumar, A.R., Suresh, H.S., Ramesh, R., Journal, S., Ecosystem, T., Change, G., May, V.M., 1995. Climate Change and Its Impact on Tropical Montane Ecosystems in Southern India. J. Biogeogr. 22, 533–536.

Sukumar, R., Ramesh, R., Pant, R.K., Rajagopalan, G., 1993. A δ13C record of late Quaternary climate change from tropical peats in southern India. Nature 364, 703–706. https://doi.org/10.1038/364703a0

Sures, B., 2008. Environmental Parasitology. Interactions between parasites and pollutants in the aquatic environment. Parasite 15, 434–438. https://doi.org/10.1051/parasite/2008153434

Sures, B., 2001. The use of fish parasites as bioindicators of heavy metals in aquatic ecosystems: A review. Aquat. Ecol. 35, 245–255. https://doi.org/10.1023/A:1011422310314

Sures, B., Nachev, M., Pahl, M., Grabner, D., Selbach, C., 2017a. Parasites as drivers of key processes in aquatic ecosystems: Facts and future directions. Exp. Parasitol. 180, 141–147. https://doi.org/10.1016/j.exppara.2017.03.011

Sures, B., Nachev, M., Selbach, C., Marcogliese, D.J., 2017b. Parasite responses to pollution: what we know and where we go in 'Environmental Parasitology.' Parasites and Vectors 10, 1–19. https://doi.org/10.1186/s13071-017-2001-3

Sutton, J.T., Castro, I., Robertson, B.C., Tompkins, D.M., Stanton, J.A.L., Jamieson, I.G., 2016. MHC genetic diversity and avian malaria prevalence in Mokoia Island saddlebacks. N. Z. J. Ecol. 40, 351–360. https://doi.org/10.20417/nzjecol.40.34

Svensson-coelho, M., Blake, J.G., Loiselle, B.A., Penrose, A.S., Parker, P.G., Ricklefs, R.E., 2013. Diversity, Prevalence, and Host specificity of Avian Plasmodium and Haemoproteus in a Western Amazon Assemblage. Ornithol. Monogr. 76, 1–47. https://doi.org/10.1525/om.2013.76.1.1.1

Svensson-Coelho, M., Ricklefs, R.E., 2011. Host phylogeography and beta diversity in avian haemosporidian (Plasmodiidae) assemblages of the Lesser Antilles. J. Anim. Ecol. 80, 938–946. https://doi.org/10.1111/j.1365-2656.2011.01837.x

Swetnam, D., Widen, S.G., Wood, T.G., Reyna, M., Wilkerson, L., Debboun, M., Symonds, D.A., Mead, D.G., Beaty, B.J., Guzman, H., Tesh, R.B., Barrett, A.D.T., 2018. Terrestrial bird migration and west nile virus circulation, United States. Emerg. Infect. Dis. 24, 2184–2194. https://doi.org/10.3201/eid2412.180382

Taberlet, P., Coissac, E., Hajibabaei, M., Rieseberg, L.H., 2012. Environmental DNA. Mol. Ecol. 21, 1789–1793. https://doi.org/10.1111/j.1365-294X.2012.05542.x

Tachibana, S.-I., Sullivan, S.A., Kawai, S., Nakamura, S., Kim, H.R., Goto, N., Arisue, N., Palacpac, N.M.Q., Honma, H., Yagi, M., Tougan, T., Katakai, Y., Kaneko, O., Mita, T., Kita, K., Yasutomi, Y., Sutton, P.L., Shakhbatyan, R., Horii, T., Yasunaga, T., Barnwell, J.W., Escalante, A.A., Carlton, J.M., Tanabe, K., 2012. Plasmodium cynomolgi genome sequences provide insight into Plasmodium vivax and the monkey malaria clade. Nat. Genet. 44, 1051–1055. https://doi.org/10.1038/ng.2375

Talavera, G., Castresana, J., 2007. Improvement of phylogenies after removing divergent and ambiguously aligned blocks from protein sequence alignments. Syst. Biol. https://doi.org/10.1080/10635150701472164

Tanaka, R., Hino, A., Tsai, I.J., Palomares-Rius, J.E., Yoshida, A., Ogura, Y., Hayashi, T., Maruyama, H., Kikuchi, T., 2014. Assessment of helminth biodiversity in wild rats using 18S rDNA based metagenomics. PLoS One 9, e110769. https://doi.org/10.1371/journal.pone.0110769

Telford, S., 2009. Hemoparasites of the Reptilia : color atlas and text. CRC Press.

Tella, J.L., 2002. The evolutionary transition to coloniality promotes higher blood parasitism in birds 15, 32–41.

Telleria, E.L., Martins-Da-Silva, A., Tempone, A.J., Traub-Cseko, Y.M., 2018. Leishmania, microbiota and sand fly immunity. Parasitology 145, 1336–1353. https://doi.org/10.1017/S0031182018001014

Tellez, M., Merchant, M., 2015. Biomonitoring heavy metal pollution using an aquatic apex

predator, the American Alligator, and its Parasites. PLoS One 10, 1–18. https://doi.org/10.1371/journal.pone.0142522

Teotônio, I.M.S.N., Dias, N., Hagström-Bex, L., Nitz, N., Francisco, A.F., Hecht, M., 2019. Intestinal microbiota – A modulator of the Trypanosoma cruzi-vector-host triad. Microb. Pathog. 103711. https://doi.org/10.1016/j.micpath.2019.103711

Thézé, J., Li, T., du Plessis, L., Bouquet, J., Kraemer, M.U.G., Somasekar, S., Yu, G., de Cesare, M., Balmaseda, A., Kuan, G., Harris, E., Wu, C. hsi, Ansari, M.A., Bowden, R., Faria, N.R., Yagi, S., Messenger, S., Brooks, T., Stone, M., Bloch, E.M., Busch, M., Muñoz-Medina, J.E., González-Bonilla, C.R., Wolinsky, S., López, S., Arias, C.F., Bonsall, D., Chiu, C.Y., Pybus, O.G., 2018. Genomic Epidemiology Reconstructs the Introduction and Spread of Zika Virus in Central America and Mexico. Cell Host Microbe 23, 855-864.e7. https://doi.org/10.1016/j.chom.2018.04.017

Thogmartin, W.E., Sanders-Reed, C.A., Szymanski, J.A., McKann, P.C., Pruitt, L., King, R.A., Runge, M.C., Russell, R.E., 2013. White-nose syndrome is likely to extirpate the endangered Indiana bat over large parts of its range. Biol. Conserv. 160, 162–172. https://doi.org/10.1016/j.biocon.2013.01.010

Thomsen, P.F., Willerslev, E., 2015. Environmental DNA - An emerging tool in conservation for monitoring past and present biodiversity. Biol. Conserv. 183, 4–18. https://doi.org/10.1016/j.biocon.2014.11.019

Tompkins, D.M., Dunn, A.M., Smith, M.J., Telfer, S., 2011. Wildlife diseases: From individuals to ecosystems. J. Anim. Ecol. 80, 19–38. https://doi.org/10.1111/j.1365-2656.2010.01742.x

Tompkins, D.M., Gleeson, D.M., 2006. Relationship between avian malaria distribution and an

exotic invasive mosquito in new zealand. J. R. Soc. New Zeal. 36, 51–62. https://doi.org/10.1080/03014223.2006.9517799

Tompkins, D.M., Sainsbury, A.W., Nettleton, P., Buxton, D., Gurnell, J., 2002. Parapoxvirus causes a deleterious disease in red squirrels associated with UK population declines. Proc. R. Soc. B Biol. Sci. 269, 529–533. https://doi.org/10.1098/rspb.2001.1897

Tompkins, D.M., White, A.R., Boots, M., 2003. Ecological replacement of native red squirrels by invasive greys driven by disease. Ecol. Lett. 6, 189–196. https://doi.org/10.1046/j.1461-0248.2003.00417.x

Torchin, M.E., Lafferty, K.D., Dobson, A.P., McKenzie, V.J., Kuris, A.M., 2003. Introduced species and their missing parasites. Nature 421, 628–630. https://doi.org/10.1038/nature01346

Townsend, A.K., Clark, A.B., McGowan, K.J., Buckles, E.L., Miller, A.D., Lovette, I.J., 2009. Disease-mediated inbreeding depression in a large, open population of cooperative crows. Proc. R. Soc. B 276, 2057–2064. https://doi.org/10.1098/rspb.2008.1852

Townsend, A.K., Taff, C.C., Wheeler, S.S., Weis, A.M., Hinton, M.G., Jones, M.L., Logsdon, R.M., Reisen, W.K., Freund, D., Sehgal, R.N.M., Saberi, M., Suh, Y.H., Hurd, J., Boyce, W.M., 2018. Low heterozygosity is associated with vector-borne disease in crows. Ecosphere 9, e02407. https://doi.org/10.1002/ecs2.2407

Trewby, H., Nadin-Davis, S.A., Real, L.A., Biek, R., 2017. Processes underlying rabies virus incursions across US–Canada border as revealed by whole-genome phylogeography. Emerg. Infect. Dis. 23, 1454–1461. https://doi.org/10.3201/eid2309.170325

Tschirren, B., Andersson, M., Scherman, K., Westerdahl, H., Mittl, P.R., Raberg, L., 2013.

Polymorphisms at the innate immune receptor TLR2 are associated with Borrelia infection in a wild rodent population. Proc Biol Sci 280, 20130364. https://doi.org/10.1098/rspb.2013.0364

Turnbaugh, P.J., Ley, R.E., Hamady, M., Fraser-Liggett, C.M., Knight, R., Gordon, J.I., 2007. The Human Microbiome Project. Nature 449, 804–810. https://doi.org/10.1038/nature06244

Turner, A.K., Begon, M., Jackson, J.A., Paterson, S., 2012. Evidence for selection at cytokine loci in a natural population of field voles (Microtus agrestis). Mol. Ecol. 21, 1632–1646. https://doi.org/10.1111/j.1365-294X.2012.05501.x

Valkiŭnas, G., 2005. Avian malaria parasites and other haemosporidia. CRC Press.

Van Dongen, S., 1999. Accuracy and power in the statistical analysis of fluctuating asymmetry: effects of between-individual heterogeneity in developmental instability. Ann. Zool. Fennici 36, 45–52.

VanderWaal, K.L., Atwill, E.R., Isbell, L.A., McCowan, B., 2014a. Linking social and pathogen transmission networks using microbial genetics in giraffe (Giraffa camelopardalis). J. Anim. Ecol. 83, 406–414. https://doi.org/10.1111/1365-2656.12137

VanderWaal, K.L., Atwill, E.R., Isbell, L.A., McCowan, B., 2014b. Quantifying microbe transmission networks for wild and domestic ungulates in Kenya. Biol. Conserv. 169, 136–146. https://doi.org/10.1016/j.biocon.2013.11.008

Vannatta, J.T., Minchella, D.J., 2018. Parasites and Their Impact on Ecosystem Nutrient Cycling. Trends Parasitol. 34, 452–455. https://doi.org/10.1016/j.pt.2018.02.007

Vidal-Martínez, V.M., Pech, D., Sures, B., Purucker, S.T., Poulin, R., 2010. Can parasites really reveal environmental impact? Trends Parasitol. 26, 44–51.

https://doi.org/10.1016/j.pt.2009.11.001

Videvall, E., 2019. Genomic Advances in Avian Malaria Research. Trends Parasitol. 35, 254–266. https://doi.org/10.1016/j.pt.2018.12.005

Videvall, E., 2018. Plasmodium parasites of birds have the most AT-rich genes of eukaryotes. Microb. genomics 4. https://doi.org/10.1099/mgen.0.000150

Videvall, E., Cornwallis, C.K., Ahrén, D., Palinauskas, V., Valkiūnas, G., Hellgren, O., 2017. The transcriptome of the avian malaria parasite Plasmodium ashfordi displays host-specific gene expression. Mol. Ecol. 26, 2939–2958. https://doi.org/10.1111/mec.14085

Videvall, E., Cornwallis, C.K., Palinauskas, V., Valkiunas, G., Hellgren, O., 2015. The avian transcriptome response to malaria infection. Mol. Biol. Evol. 32, 1255–1267. https://doi.org/10.1093/molbev/msv016

Vidya, T.N.C., Fernando, P., Melnick, D.J., Sukumar, R., 2005. Population differentiation within and among Asian elephant (Elephas maximus) populations in southern India. Heredity 94, 71. https://doi.org/10.1038/sj.hdy.6800568

Vijayakumar, S.P., Menezes, R.C., Jayarajan, A., Shanker, K., 2016. Glaciations, gradients, and geography: multiple drivers of diversification of bush frogs in the Western Ghats Escarpment. Proc. R. Soc. B Biol. Sci. 282. https://doi.org/10.1098/rspb.2016.1011

Vilaça, S.T., Grant, S.A., Beaty, L., Brunetti, C.R., Congram, M., Murray, D.L., Wilson, C.C., Kyle, C.J., 2019. Detection of spatiotemporal variation in ranavirus distribution using eDNA. Environ. DNA 1–11. https://doi.org/10.1002/edn3.59

Vincent, C.M., Sharp, N.P., 2014. Sexual antagonism for resistance and tolerance to infection in Drosophila melanogaster. Proc. R. Soc. B Biol. Sci. 281, 20140987.

Volz, E.M., Koelle, K., Bedford, T., 2013. Viral Phylodynamics. PLoS Comput. Biol. 9, e1002947. https://doi.org/10.1371/journal.pcbi.1002947

Volz, E.M., Romero-Severson, E., Leitner, T., 2017. Phylodynamic Inference across Epidemic Scales. Mol. Biol. Evol. 34, 1276–1288. https://doi.org/10.1093/molbev/msx077

Von Schantz, T., Wittzell, H., Goransson, G., Grahn, M., Persson, K., 1996. MHC genotype and male ornamentation: Genetic evidence for the Hamilton-Zuk model. Proc. R. Soc. B Biol. Sci. 263, 265–271. https://doi.org/10.1098/rspb.1996.0041

Vrancken, B., Lemey, P., Rambaut, A., Bedford, T., Longdon, B., Günthard, H.F., Suchard, M.A., 2015. Simultaneously estimating evolutionary history and repeated traits phylogenetic signal: Applications to viral and host phenotypic evolution. Methods Ecol. Evol. 6, 67–82. https://doi.org/10.1111/2041-210X.12293

Vrijenhoek, R.C., Leberg, P.L., 1991. Let' s Not Throw the Baby Out with the Bathwater : A Comment on Management for MHC Diversity in Captive Populations. Conserv. Biol. 5, 252–254.

Wang, S., Liu, C., Wilson, A.B., Zhao, N., Li, X., Zhu, W., Gao, X., Liu, X., Li, Y., 2017. Pathogen richness and abundance predict patterns of adaptive major histocompatibility complex variation in insular amphibians. Mol. Ecol. 26, 4671–4685. https://doi.org/10.1111/mec.14242

Warner, R.E., 1968. The role of introduced diseases in the extinction of the endemic Hawaiian avifauna. Condor 70, 101–120. https://doi.org/10.2307/1365954

Waters, A.P., Higgins, D.G., McCutchan, T.F., 1991. Plasmodium falciparum appears to have arisen as a result of lateral transfer between avian and human hosts. Proc. Natl. Acad. Sci.

88, 3140–3144. https://doi.org/10.1073/pnas.88.8.3140

Webb, C.O., 2000. Exploring the Phylogenetic Structure of Ecological Communities: An Example for Rain Forest Trees. Am. Nat. 156. https://doi.org/10.1086/303378

Weeks, A.R., Turelli, M., Harcombe, W.R., Reynolds, K.T., Hoffmann, A.A., 2007. From parasite to mutualist: Rapid evolution of Wolbachia in natural populations of Drosophila. PLoS Biol. 5, 0997–1005. https://doi.org/10.1371/journal.pbio.0050114

Wegner, K.M., Reusch, T.B.H., Kalbe, M., 2003. Multiple parasites are driving major histocompatibility complex polymorphism in the wild. J. Evol. Biol. 16, 224–232. https://doi.org/10.1046/j.1420-9101.2003.00519.x

Weinstein, S., Titcomb, G., Agwanda, B., Riginos, C., Young, H., 2017. Parasite responses to large mammal loss in an African savanna. Ecology 98, 1839–1848. https://doi.org/10.1002/ecy.1858

Weldon, C., Du Preez, L.H., Hyatt, A.D., Muller, R., Speare, R., 2004. Origin of the amphibian chytrid fungus. Emerg. Infect. Dis. 10, 2100. https://doi.org/10.3201/eid1012.030804

Westermann, A.J., Gorski, S.A., Vogel, J., 2012. Dual RNA-seq of pathogen and host. Nat. Rev. Microbiol. 10, 618–630. https://doi.org/10.1038/nrmicro2852

Whiles, M.R., Hall, R.O., Dodds, W.K., Verburg, P., Huryn, A.D., Pringle, C.M., Lips, K.R., Kilham, S.S., Colón-Gaud, C., Rugenski, A.T., Peterson, S., Connelly, S., 2012. Disease-Driven Amphibian Declines Alter Ecosystem Processes in a Tropical Stream. Ecosystems 16, 146–157. https://doi.org/10.1007/s10021-012-9602-7

White, L.A., Forester, J.D., Craft, M.E., 2017. Using contact networks to explore mechanisms of parasite transmission in wildlife. Biol. Rev. 92, 389–409. https://doi.org/10.1111/brv.12236

Whitehorn, P.R., Tinsley, M.C., Brown, M.J.F., Darvill, B., Goulson, D., 2011. Genetic diversity, parasite prevalence and immunity in wild bumblebees. Proc. R. Soc. B Biol. Sci. 278, 1195–1202. https://doi.org/10.1098/rspb.2010.1550

WHO, 2016. WHO: Vector-borne diseases. URL http://www.who.int/mediacentre/factsheets/fs387/en (accessed 6.10.17).

Wilder, S.M., Raubenheimer, D., Simpson, S.J., 2016. Moving beyond body condition indices as an estimate of fitness in ecological and evolutionary studies. Funct. Ecol. 30, 108–115. https://doi.org/10.1111/1365-2435.12460

Wilman, H., Belmaker, J., Simpson, J., de la Rosa, C., Rivadeneira, M.M., Jetz, W., 2014. EltonTraits 1.0: Species-level foraging attributes of the world's birds and mammals. Ecology. https://doi.org/10.1890/13-1917.1

Wilmers, C.C., Post, E., Peterson, R.O., Vucetich, J.A., 2006. Predator disease out-break modulates top-down, bottom-up and climatic effects on herbivore population dynamics. Ecol. Lett. 9, 383–389. https://doi.org/10.1111/j.1461-0248.2006.00890.x

Wilson, M., Glaser, K.C., Adams-Fish, D., Boley, M., Mayda, M., Molestina, R.E., 2015. Development of droplet digital PCR for the detection of Babesia microti and Babesia duncani. Exp. Parasitol. 149, 24–31. https://doi.org/10.1016/j.exppara.2014.12.003

Winter, M., Devictor, V., Schweiger, O., 2013. Phylogenetic diversity and nature conservation: Where are we? Trends Ecol. Evol. 28, 199–204. https://doi.org/10.1016/j.tree.2012.10.015

Winternitz, J.C., Minchey, S.G., Garamszegi, L.Z., Huang, S., Stephens, P.R., Altizer, S., 2013. Sexual selection explains more functional variation in the mammalian major histocompatibility complex than parasitism. Proc. R. Soc. B Biol. Sci. 280, 20131605.

https://doi.org/10.1098/rspb.2013.1605

Wood, C.L., Johnson, P.T.J., 2015. A world without parasites: Exploring the hidden ecology of infection. Front. Ecol. Environ. 13, 425–434. https://doi.org/10.1890/140368

Woodworth, B.L., Atkinson, C.T., LaPointe, D.A., Hart, P.J., Spiegel, C.S., Tweed, E.J., Henneman, C., LeBrun, J., Denette, T., DeMots, R., Kozar, K.L., Triglia, D., Lease, D., Gregor, A., Smith, T., Duffy, D., 2005. Host population persistence in the face of introduced vector-borne diseases: Hawaii amakihi and avian malaria. Proc. Natl. Acad. Sci. 102, 1531–1536. https://doi.org/10.2749/101686610793557645

Woolhouse, M.E.J., Haydon, D.T., Antia, R., 2005. Emerging pathogens: The epidemiology and evolution of species jumps. Trends Ecol. Evol. 20, 238–244. https://doi.org/10.1016/j.tree.2005.02.009

Worby, C.J., Lipsitch, M., Hanage, W.P., 2017. Shared Genomic Variants: Identification of Transmission Routes Using Pathogen Deep-Sequence Data. Am. J. Epidemiol. 186, 1209–1216. https://doi.org/10.1093/aje/kwx182

Wyatt, K.B., Campos, P.F., Gilbert, M.T.P., Kolokotronis, S.-O., Hynes, W.H., DeSalle, R., Daszak, P., MacPhee, R.D.E., Greenwood, A.D., 2008. Historical Mammal Extinction on Christmas Island (Indian Ocean) Correlates with Introduced Infectious Disease. PLoS One 3, e3602. https://doi.org/10.1371/journal.pone.0003602

Wylie, T.N., Wylie, K.M., Herter, B.N., Storch, G.A., Author, C., Louis, S., 2015. Enhanced Virome Sequencing Using Targeted Sequence Capture Enhanced virome sequencing using sequence capture. Genome Res. 4, 1910–1920. https://doi.org/10.1101/gr.191049.115.Freely

Yap, T.A., Nguyen, N.T., Serr, M., Shepack, A., Vredenburg, V.T., 2017. Batrachochytrium salamandrivorans and the Risk of a Second Amphibian Pandemic. Ecohealth 14, 851–864. https://doi.org/10.1007/s10393-017-1278-1

Zehtindjiev, P., Ilieva, M., Westerdahl, H., Hansson, B., Valkiunas, G., Bensch, S., 2008. Dynamics of parasitemia of malaria parasites in a naturally and experimentally infected migratory songbird, the great reed warbler *Acrocephalus arundinaceus*. Exp. Parasitol. 119, 99–110. https://doi.org/10.1016/j.exppara.2007.12.018

Zhang, L., Wu, Q., Hu, Y., Wu, H., Wei, F., 2015. Major histocompatibility complex alleles associated with parasite susceptibility in wild giant pandas. Heredity 114, 85–93. https://doi.org/10.1038/hdy.2014.73

APPENDICES

APPENDIX A

ELECTRONIC SUPPLEMENTARY MATERIAL FOR CHAPTER 3

1. Methods

1.1. Field sampling

Field sampling was conducted at 7-14 sites across four major geographical regions in the southern 600 km mountain range of the Western Ghats spanning elevation 100-2500m (figure 3.1; electronic supplementary material, Appendix A S3.1). Adult birds were sampled with mist-nets (12m*2m) in the pre-monsoon, pre-breeding season (January-May) during 2011-2013. Each geographical region corresponded to the sky-island group separated by three biogeographical barriers—Chaliyar River valley between I (Bababudan & Banasura hills) & II (Nilgiri hills), Palghat Gap between II & III (Anamalai-Palni-Highwavies Hills) and Shencottah Gap between III & IV (Ashambu hills) (figure 3.1). We collected blood samples (50-100 μl) from the ulnar vein of the bird with a heparinized micro-hematocrit capillary tube and immediately stored in Queen's blood lysis buffer (Seutin et al., 1991). Samples were stored at room temperature during field season (2-3 weeks) and later transferred at -20 ℃ in the laboratory until further analyses. Detailed sampling methods and post-capture procedures are described in an earlier study (Robin et al., 2010).

1.2. Parasite detection and identification

A nested PCR approach was employed to amplify the partial mitochondrial cytochrome *b* gene (478bp) of avian haemosporidian parasites (Hellgren et al., 2004). Parasite DNA was amplified in a 10 µl reaction containing 1 µl of template DNA, 1 µl of each primer (0.2 µM), 4µl of Multiplex PCR Master Mix (Qiagen, Germany), and 4 µl of water in an Eppendorf thermocycler. PCR conditions included an initial denaturation at 95 ˚C for 15 min, followed by 40 cycles at 94 ˚C for 30 s, T_a for 30 s, 72 ˚C for 40 s, followed by a final extension at 72 ˚C for 15 min and hold at 4 ˚C. The reference genomic DNA of *Plasmodium relictum* provided by National Institute of Malaria Research, New Delhi, India, was used as a positive control for all PCR reactions. PCR products were checked visually by running 2 µl of the amplified product on a 2% agarose gel stained with 1X Gel Red. Prior to sequencing, amplified products were purified using a mix of ExoSAP (USB Corporation, USA) and sequenced in both forward and reverse direction using BigDye v. 3.1 (Applied Biosystems, Germany) on an ABI 3130 automated DNA sequencer. Paired DNA sequences were aligned, trimmed and assembled in Geneious 9.1.5 (Kearse et al., 2012). Sequences containing one or more double peaks were scored as 'multiple infection' and discarded from further analyses. Each parasite lineage was compared with sequences available in GenBank and MalAvi databases (Bensch et al., 2009) to identify the genus. Lineages differing by at least 1bp was considered as a unique parasite lineage (Bensch et al., 2004).

1.3. Phylogenetic analyses

To assess phylogenetic relationships among 18 *Plasmodium* spp. and 29 *Haemoproteus* spp. lineages isolated from the Shola Sky Island bird community, we used 478 bp of cytochrome

b sequence data (described above). We then performed a Bayesian phylogenetic analyses in MrBayes ver 3.1.2 (Ronquist and Huelsenbeck, 2003). The best nucleotide substitution model for phylogenetic analysis was determined by jModelTest based on AIC values (Darriba et al., 2012; Posada, 2008). Two independent runs, each with four Markov chain Monte Carlo (MCMC) chains were performed, using the GTR+G+I substitution model, for 20 million generations and sampling every 500th generation by discarding 25% of the samples as burn-in. The stationarity of the chains across the two runs was examined by visualizing the log-likelihood plots and effective sample size (ESS>200). Adequate convergence of runs was assessed by evaluating the average standard deviation of split frequencies (<0.01) and Potential Scale Reduction Factor (PSRF) of summarized parameters (PSRF close to 1).

To assess parasite phylogenetic relationships at the global community level, we built a phylogenetic tree with cytochrome b sequence data for *Haemoproteus* spp. (19) and *Plasmodium* spp. (18) lineages recovered from the Shola Sky Island bird community and *Plasmodium* spp. (957) and *Haemoproteus* spp. (1025) lineages from the MalAvi database (Bensch et al., 2009) (accessed February 2018). We created a subset of the global data to include only parasites sequences of more than 300 bp of cytb fragment to ensure the availability of adequate phylogenetic information, in terms of the number of sequences and sequence data, for comparative analysis of all parasite lineages. Bayesian phylogenetic analysis was implemented in MrBayes ver 3.1.2 (Ronquist and Huelsenbeck, 2003) with run conditions same as described above for the Shola Sky Island avian haemosporidians phylogeny. We also built Bayesian host phylogeny in MrBayes ver 3.1.2 (Ronquist and Huelsenbeck, 2003) using the cytochrome b sequence data (1143bp) for 28 bird species from an earlier study (Robin et al., 2015b, 2017),

with similar parameters and run conditions as described above. All analyses were performed on the CIPRES Science Gateway (Miller et al., 2010) and phylogenetic trees were visualized in FigTree 1.4.0 (http://tree.bio.ed.ac.uk/ software/figtree/) (Rambaut, 2012).

1.4. Rarefaction analyses

To ensure adequate sampling, we calculated the rarefaction curves of expected phylogenetic diversity (mean and variance) for multiple values of sampling effort (i.e., number of individuals sampled) for host species and parasite lineages. Expected phylogenetic diversity was calculated using the exact analytical formulation proposed by Nipperess and Matesen (Nipperess and Matsen, 2013) and was implemented in the R package PDcalc v0.3.2.9 (Nipperess and Matsen, 2013).

1.5. Host specificity analyses

We then quantified host specialization for each haemosporidian lineage (infecting ≥2 host species) by measuring the phylospecificity index—mean phylogenetic distance (MPD) between all possible pairs of infected host species (Webb, 2000). We estimated MPD by taking into account the frequency of infected hosts and phylogenetic relationships among the infected host species (Poulin et al., 2011). We then calculated standardized effect sizes of the MPD values (SES.MPD) as the difference between the observed MPD values (MPD_{obs}) and expected MPD values (MPD_{exp}) under a null model, divided by the standard deviation of the MPD values obtained from the null data. We carried out 100,000 randomizations to produce a null distribution of MPD values by keeping the host topology intact and randomizing tip labels across the host phylogeny using the function ‘mpd’ and ‘ses.mpd’ as implemented in the R package

picante (Kembel et al., 2010). For each parasite lineage, we assessed statistical significance by calculating the probability of obtaining MPD_{exp} value less than or equal to MPD_{obs} value.

1.6. Host-parasite co-phylogenetic analyses

We visually assessed phylogenetic congruence between the host and parasite phylogenetic trees by constructing a cophylogenetic tanglegram using TreeMap 3.0 (Charleston and Robertson, 2002). We then statistically tested for host-parasite phylogenetic congruence using a distance based co-phylogenetic analyses in Procrustean Approach to Cophylogeny (PACo) (Balbuena et al., 2013). PACo tests the dependence of parasite phylogeny onto the host phylogeny and requires three matrices— patristic/genetic distance matrices estimated from host phylogenies, parasite phylogenies and host-parasite association matrix to calculate the global goodness-of-fit statistic of congruence between the two trees. We ran 10 million randomizations of the host-parasite association matrix in PACo program available with the R packages ape v3.5 (Bolker et al., 2014) and vegan v2.4.1 (Oksanen et al., 2016).

We determined the type and frequency of different evolutionary scenarios driving the host-parasite coevolutionary patterns in JANE v4.01 (Conow et al., 2010) and CoRe-PA (Merkle et al., 2010). Both event-based methods infer host-parasite coevolutionary history based on host and parasite phylogenies by evaluating the occurrence of a set of evolutionary events— co-speciation (Co), duplication (Du), host switch (Hs), sorting or loss of parasite lineages (So). While JANE assigns an a priori cost for each evolutionary event, CoRe-PA does not require an a priori assignment of cost values and computes a cost minimal reconstruction by assuming that event costs are inversely related to the relative frequency of the corresponding event. We used the default cost setting in JANE, with 0 for Co, 1 for Du, 2 for Du and Hs, 1 for So, and 1 for

failure to diverge and set the genetic algorithm parameters at 100 generations and a population size of 500 to obtain the optimal least cost solutions. We then randomized tip associations 500 times to test whether the inferred reconstruction cost was significantly lower than expected by chance. Furthermore, in CoRe-PA, we used the default cost method to automatically calculate cost values for each evolutionary event and then used the optimized cost values to reconstruct host-parasite coevolutionary history. We randomized host-parasite associations 1000 times to test whether the observed numbers of each evolutionary event were significantly higher than expected by chance.

2. Tables

Table S3.1. Sampling locations in each geographical region identified by their geographical coordinates and mean elevation(m) along with the number of trapping sites and samples collected to study avian haemosporidians in the Shola Sky Island bird communities in Western Ghats (India) during 2011-2013. Each geographical region: I (Bababudan & Banasura hills), II (Nilgiri hills), III (Anamalai-Palni-Highwavies Hills), IV (Ashambu hills) corresponds to the major Sky Island group separated by three biogeographical barriers—Chaliyar Gap, Palghat Gap and Shencottah Gap.

Geographical Region	Location name	Number of Trapping sites	Sample size	Latitude	Longitude	Elevation (m)
I	Vellarimala	1	21	11.47	76.13	1821.00
I	Chembra	1	29	11.53	76.09	1731.00
I	Banasura	4	61	11.67	75.93	1737.00
I	Ambalapara	6	150	11.94	75.94	1497.83
II	Sispara	4	86	11.18	76.47	2105.00
II	Silent valley	10	147	11.19	76.54	1449.70
II	Ooty	1	15	11.39	76.68	2246.00
III	Periyar	3	25	9.56	77.14	880.67
III	High Wavies	4	141	9.58	77.33	1573.75
III	Munnar	3	189	10.13	77.09	2065.00
III	Kodi	6	99	10.23	77.43	2096.00
III	Grasshills	1	7	10.33	77.02	1621.00
IV	Peppara	8	207	8.68	77.18	960.50

Table S3.2. Avian haemosporidian parasite prevalence across the Shola Sky Island bird community in the Western Ghats sampled during 2011-2013. Reported are the common and scientific names of host species, host species code, sample size (N_{TOT}), number of individuals infected with haemosporidian parasites (N_{INF}), proportion of infected individuals for *Plasmodium* spp. (P), *Haemoproteus* spp. (H) and Multiple infections (MI). Lineages represent the unique genetic lineages in each host species identified from GenBank and MalAvi databases.

Scientific Name	**Common name**	**Species Code**	**N_{TOT}**	**N_{INF}**	**Prevalence (%)**			**Unique lineages**	
					P	**H**	**MI**	**P**	**H**
*Columba elphinstonii*ǂ	Nilgiri wood pigeon	COLELP	6	2	0.00	33.33	0.00	0	1
*Montecincla meridionalis*ǂ	Ashambu chilappan	MONMER	36	19	2.78	44.44	5.56	0	3
*Montecincla jerdoni*ǂ	Banasura chilappan	MONJER	21	11	4.76	47.62	0.00	1	1
*Montecincla cachinnans*ǂ	Nilgiri chilappan	MONCAC	32	19	3.13	46.88	9.38	1	3
*Montecincla fairbanki*ǂ	Palani chilappan	MONFAI	102	71	2.94	52.94	13.73	3	3
Alcippe poioicephala	Brown cheeked fulvetta	ALCPOI	57	25	5.26	33.33	5.26	2	6
*Garrulax delesserti*ǂ	Wayanad laughingthrush	GARDEL	38	30	13.16	44.74	21.05	2	4
*Schoenicola platyurus*ǂ	Broad- tailed grassbird	SCHPLA	7	0	0.00	0.00	0.00	0	0

Scientific Name	Common name	Species Code	N_{TOT}	N_{INF}	Prevalence (%)			Unique lineages	
					P	H	MI	P	H
Anthus nilghiriensis	Nilgiri pipit	ANTNIL	16	0	0.00	0.00	0.00	0	0
Anthus rufulus	Paddyfield pipit	ANTRUF	5	1	20.00	0.00	0.00	1	0
Sholicola ashambuensis‡	Ashambu shortwing	SHOASH	21	2	0.00	9.52	0.00	0	1
Ficedula nigrorufa‡	Black and orange flycatcher	FICNIG	106	11	1.89	7.55	0.94	2	2
Eumyias albicaudatus‡	Nilgiri flycatcher	EUMALB	43	3	4.65	0.00	2.33	2	0
Saxicola caprata	Pied bushchat	SAXCAP	17	2	5.88	5.88	0.00	1	1
Sholicola major‡	Rufous bellied shortwing	SHOMAJ	86	19	1.16	20.93	0.00	1	3
Cyornis pallipes‡	White bellied blue flycatcher	CYOPAL	17	5	0.00	29.41	0.00	0	1
Sholicola albiventris‡	White bellied shortwing	SHOALB	100	34	1.00	32.00	1.00	1	2
Pellorneum ruficeps	Puff- throated babbler	PELRUF	27	1	3.70	0.00	0.00	1	0
Hypsipetes leucocephalus	Black bulbul	HYPLEU	58	45	12.07	51.72	13.79	1	3
Pycnonotus jocosus	Red-whiskered bulbul	PYCJOC	26	1	0.00	3.85	0.00	0	1

Scientific Name	Common name	Species Code	N_{TOT}	N_{INF}	Prevalence (%)			Unique lineages	
					P	H	MI	P	H
Iole indica	Yellow browed bulbul	IOLIND	25	12	16.00	12.00	16.00	4	1
Culicicapa ceylonensis	Grey- headed flycatcher	CULCEY	29	0	0.00	0.00	0.00	0	0
Rhopocichla atriceps	Dark- fronted babbler	RHOATR	34	0	0.00	0.00	0.00	0	0
Pomatorhinus horsfieldii	Scimitar babbler	POMHOR	37	8	2.70	13.51	5.41	1	1
Turdus merula	Blackbird	TURMER	86	56	29.07	6.98	23.26	8	3
Zoothera citrina	Orange-headed ground thrush	ZOOCIT	10	5	30.00	0.00	20.00	2	0
Zoothera dauma	Scaly thrush	ZOODAU	17	11	0.00	17.65	35.29	1	1
Zosterops palpebrosus	Oriental white eye	ZOSPAL	118	97	0.85	77.12	5.08	0	4
			1177	**490**	**13.06**	**68.98**	**16.53**	**35**	**45**

[1]Western Ghats endemic species

Table S3.3. Host species infected and GenBank accession numbers (this study) for the *Plasmodium* spp. and *Haemoproteus* spp. lineages isolated from the Shola Sky Island bird community. Lineages were matched with the MalAvi database and reference lineage names (percent similarity values) are shown. Lineages that matched 100% with MalAvi reference lineages are marked in bold.

Parasite lineage	**Host species infected**	**MalAvi reference lineage (% similarity)**	**GenBank Accession No.**
Plasmodium spp.			
WG_P_MSP01	*Sholicola albiventris, Iole indica*	CXPIP11 (100%)	
WG_P_ANTRUF01	*Anthus rufulus*	PBPIP1 (100%)	
WG_P_SAXCAP01	*Saxicola caprata*	GALLUS01 (100%)	
WG_P_MSP02	*Zoothera dauma, Zoothera citrina, Iole indica, Ficedula nigrorufa, Eumyias albicaudatus, Alcippe poioicephala*	ORW1 (100%)	
WG_P_MSP03	*Sholicola major, Pellorneum ruficeps, Pomatorhinus horsfieldii, Montecincla fairbanki, Montecincla jerdoni, Hypsipetes leucocephalus, Turdus merula, Iole indica, Garrulax delesserti, Ficedula nigrorufa, Alcippe poioicephala*	NILSUN01 (100%)	
WG_P_MONCAC01	*Montecincla cachinnans*	GARPEC01 (98%)	MK493380
WG_P_TURMER01	*Turdus merula*	GRW06 (100%)	
WG_P_TURMER02	*Turdus merula*	AFTRU4 (99%)	MK493396
WG_P_TURMER03	*Turdus merula*	AFTRU5 (99%)	MK493392
WG_P_TURMER07	*Turdus merula*	FANTAIL01 (100%)	
WG_P_TURMER04	*Turdus merula*	PROCAF01 (99%)	MK493393

Parasite lineage	**Host species infected**	**MalAvi reference lineage (% similarity)**	**GenBank Accession No.**
WG_P_TURMER05	*Turdus merula*	PROCAF01 (98%)	MK493394
WG_P_TURMER06	*Turdus merula*	FORCOL05 (98%)	MK493395
WG_P_MSP04	*Montecincla fairbanki, Iole indica*	CYAOLI11 (95%)	MK493378
WG_P_MONFAI02	*Montecincla fairbanki*	PACCAL01 (99%)	MK493382
WG_P_ZOOCIT02	*Zoothera citrina*	FORCOL05 (97%)	MK493397
WG_P_GARDEL02	*Garrulax delesserti*	ALMOR01 (99%)	MK493375
WG_P_EUMALB02	*Eumyias albicaudatus*	GRW04 (100%)	
Haemoproteus spp.			
WG_H_ZOODAU01	*Zoothera dauma*	ZOOLUN01 (99%)	MK493398
WG_H_MSP01	*Saxicola caprata, Montecincla fairbanki, Montecincla cachinnans, Montecincla meridionalis, Montecincla jerdoni*	CUKI1 (99%)	MK493384
WG_H_MSP02	*Ficedula nigrorufa, Sholicola albiventris, Sholicola ashambuensi, Sholicola major*	COLL2- (100%)	
WG_H_PYNJOC01	*Pycnonotus jocosus*	BUL2 (99%)	MK493387
WG_H_POMHOR01	*Pomatorhinus horsfieldii*	AFR059 (97%)	MK493386
WG_H_IOLIND01	*Iole indica*	HYPHI22 (99%)	MK493377
WG_H_CYOPAL01	*Cyornis pallipes*	COLL2 (99%)	MK493373
WG_H_COLELP01	*Columba elphinstonii*	STRTUR01 (99%)	MK493372
WG_H_MSP03	*Alcippe poioicephala, Garrulax delesserti*	HAECOL1 (100%)	
WG_H_MSP04	*Alcippe poioicephala, Garrulax delesserti, Montecincla meridionalis*	COLL2 (99%)	MK493368

Parasite lineage	**Host species infected**	**MalAvi reference lineage (% similarity)**	**GenBank Accession No.**
WG_H_MSP05	*Alcippe poioicephala, Garrulax delesserti, Montecincla fairbanki, Ficedula nigrorufa, Zosterops palpebrosus*	AFR137 (97%)	MK493374
WG_H_ALCPOI04	*Alcippe poioicephala*	TUMIG07 (99%)	MK493369
WG_H_ALCPOI05	*Alcippe poioicephala*	COLL2 (99%)	MK493370
WG_H_ALCPOI06	*Alcippe poioicephala*	TUMIG07 (99%)	MK493371
WG_H_MSP06	*Zosterops palpebrosus, Sholicola major*	ZOSLAT04 (99%)	MK493399
WG_H_ZOSPAL03	*Zosterops palpebrosus*	ZOSLAT04 (99%)	MK493400
WG_H_ZOSPAL04	*Zosterops palpebrosus*	YEWE2 (99%)	MK493401
WG_H_MSP07	*Garrulax delesserti, Sholicola albiventris*	DENMAG01 (98%)	MK493388
WG_H_TURMER01	*Turdus merula*	TURDUS2 (99%)	MK493390
WG_H_TURMER02	*Turdus merula*	TURDUS2 (99%)	MK493391
WG_H_TURMER03	*Turdus merula*	TUCHR01 (100%)	
WG_H_HYPLEU01	*Hypsipetes leucocephalus*	HYPHI07 (100%)	
WG_H_HYPLEU02	*Hypsipetes leucocephalus*	HYPHI04 (99%)	MK493376
WG_H_HYPLEU03	*Hypsipetes leucocephalus*	HYPHI04 (100%)	
WG_H_SHOMAJ03	*Sholicola major*	PYCCYA01 (99%)	MK493389
WG_H_MONMER02	*Montecincla meridionalis*	ROFI1 (98%)	MK493385
WG_H_MONFAI02	*Montecincla fairbanki*	ROFI1 (98%)	MK493383
WG_H_MONCAC01	*Montecincla cachinnans*	CATUST22 (99%)	MK493379
WG_H_MONCAC03	*Montecincla cachinnans*	DENMAG01 (99%)	MK493381

Table S3.4. Shannon diversity interactions index (H2) for each *Plasmodium spp.* lineage based on the number and evenness of hosts infected. Significance was assessed by randomizing network interactions to test if the difference in observed vs. expected H2 (ΔH2) were different from zero. Statistically significant results ($P < 0.05$) are in bold.

Lineage	**H2**	**ΔH2**	***P***
P_ANTRUF01	0.000	-0.794	0.189
P_EUMALB02	0.000	-0.786	0.200
P_GARDEL02	0.000	-0.800	0.197
P_MONCAC01	0.000	-0.818	0.174
P_MONFAI02	0.000	-0.800	0.170
P_MSP01	0.693	-0.103	0.487
P_MSP02	1.748	0.942	**0.016**
P_MSP03	2.246	1.451	**0.001**
P_MSP04	0.693	-0.124	0.476
P_SAXCAP01	0.000	-0.800	0.185
P_TURMER01	0.000	-0.787	0.204
P_TURMER02	0.000	-0.816	0.183
P_TURMER03	0.000	-0.842	0.183
P_TURMER04	0.000	-0.819	0.173
P_TURMER05	0.000	-0.816	0.174
P_TURMER06	0.000	-0.790	0.195
P_TURMER07	0.000	-0.791	0.200
P_ZOOCIT02	0.000	-0.791	0.207

Table S3.5. Shannon diversity interactions index (H2) for each *Haemoproteus spp.* lineage based on the number and evenness of hosts infected. Significance was assessed by randomizing network interactions to test if the difference in observed vs. expected H2 (ΔH2) were different from zero. Statistically significant results ($P < 0.05$) are in bold.

Lineage	**H2**	**ΔH2**	***P***
H_ALCPOI04	0.000	-1.704	**0.002**
H_ALCPOI05	0.000	-1.686	**0.002**
H_ALCPOI06	0.000	-1.677	**0.002**
H_COLELP01	0.000	-1.691	**0.002**
H_CYOPAL01	0.000	-1.695	**0.001**
H_HYPLEU01	0.000	-1.686	**0.002**
H_HYPLEU02	0.000	-1.689	**0.004**
H_HYPLEU03	0.000	-1.688	**0.001**
H_IOLIND01	0.000	-1.684	**0.001**
H_MONCAC01	0.000	-1.693	**0.002**
H_MONCAC03	0.000	-1.693	**0.001**
H_MONFAI02	0.000	-1.678	**0.001**
H_MONMER02	0.000	-1.685	**0.001**
H_MSP01	1.439	-0.260	0.168
H_MSP02	1.064	-0.630	**0.035**
H_MSP03	0.693	-1.014	**0.006**
H_MSP04	0.566	-1.123	**0.005**
H_MSP05	0.953	-0.734	**0.020**
H_MSP06	0.065	-1.631	**0.002**
H_MSP07	0.637	-1.051	**0.006**
H_POMHOR01	0.000	-1.697	**0.002**

H_PYNJOC01	0.000	-1.679	**0.001**
H_SHOMAJ03	0.000	-1.685	**0.002**
H_TURMER01	0.000	-1.682	**0.001**
H_TURMER02	0.000	-1.683	**0.001**
H_TURMER03	0.000	-1.691	**0.002**
H_ZOODAU01	0.000	-1.696	**0.001**
H_ZOSPAL03	0.000	-1.693	**0.001**
H_ZOSPAL04	0.000	-1.685	**0.001**

Table S3.6. Shannon diversity interactions index (H2) for each host species based on the number and evenness of parasite lineages infecting that host. Significance was assessed by randomizing network interactions to test if the difference in observed vs. expected H2 (ΔH2) were different from zero. Analysis were done separately for *Plasmodium spp.* and *Haemoproteus spp.* lineages. Statistically significant results ($P < 0.05$) are in bold.

Host species	*Plasmodium* spp,			*Haemoproteus* spp.		
	Observed	**Δ Value**	***P***	**Observed**	**Δ Value**	***P***
ALCPOI	0.693	-0.310	0.343	1.408	-0.440	**0.035**
ANTRUF	0.000	-1.015	0.116	--	--	--
COLELP	--	--	--	0.000	-1.848	**0.001**
CYOPAL	--	--	--	0.000	-1.824	**0.001**
EUMALB	0.693	-0.301	0.369	--	--	--
FICNIG	0.693	-0.314	0.354	0.377	-1.480	**0.001**
GARDEL	0.637	-0.384	0.192	0.734	-1.104	**0.001**
HYPLEU	0.000	-1.037	0.106	0.811	-1.018	**0.002**
IOLIND	1.332	0.312	0.365	0.000	-1.835	**0.001**
MONCAC	0.000	-0.989	0.132	0.898	-0.964	**0.002**
MONFAI	1.099	0.073	0.552	0.826	-1.015	**0.002**
MONJER	0.000	-1.021	0.106	0.000	-1.847	**0.001**
MONMER	--	--	--	0.703	-1.152	**0.001**
PELRUF	0.000	-0.983	0.139	--	--	--
POMHOR	0.000	-1.012	0.122	0.000	-1.829	**0.001**
PYCJOC	--	--	--	0.000	-1.836	**0.001**
SAXCAP	0.000	-1.021	0.110	0.000	-1.836	**0.001**
SHOALB	0.000	-0.996	0.131	0.139	-1.696	**0.001**
SHOASH	--	--	--	0.000	-1.841	**0.001**

SHOMAJ	0.000	-1.000	0.133	0.426	-1.399	**0.001**
TURMER	1.715	0.737	**0.046**	0.868	-0.968	**0.003**
ZOOCIT	0.562	-0.427	0.161	--	--	--
ZOODAU	0.000	-1.032	0.114	0.000	-1.843	**0.001**
ZOSPAL	--	--	--	0.390	-1.454	**0.001**

Table S3.7. Mean phylogenetic distance (MPD) weighted by frequency of hosts infected and Standardized effect size of the mean phylogenetic distance (SES.MPD) for *Plasmodium spp.* and *Haemoproteus spp.* lineages infecting ≥ 2 host species. Host phylogenetic tree tip labels were randomized to create null models and assess significance level**.** Statistically significant results ($P < 0.05$) are in bold.

Parasite	**Lineage**	**No. of host species**	**MPD**	**SES.MPD**	***P***
Plasmodium spp.	P_MSP01	2	0.313	-0.175	0.553
	P_MSP02	6	0.444	-0.666	0.338
	P_MSP03	11	0.443	-1.090	0.067
	P_MSP04	2	0.235	-0.518	0.248
Haemoproteus spp.	H_MSP01	5	0.107	-1.930	**0.000**
	H_MSP02	4	0.092	-1.573	**0.001**
	H_MSP03	2	0.160	-0.852	0.093
	H_MSP04	3	0.093	-1.037	**0.026**
	H_MSP05	5	0.179	-0.969	**0.036**
	H_MSP06	2	0.015	-0.165	0.558
	H_MSP07	2	0.278	-0.165	0.558

Table S3.8. Results of the cophylogenetic analyses in CoRe-PA program. The event costs approximated by CoRe-PA were used to reconstruct optimal co-evolutionary scenarios (Co-speciation- Co, Sorting- So, Duplication- Du, Host-switching- Hs) and randomization tests were performed to assess significance level (P <0.05; bold).

Parasite	**Event Costs**				**Events found**				**Total cost**
	Co	**So**	**Du**	**Hs**	**Co**	**So**	**Du**	**Hs**	
Plasmodium spp.	0.2	0.03	0.2	0.54	6	40	10	1	5.38
Haemoproteus spp.	0.2	0.07	0.11	0.61	**9**	26	16	3	7.33

Table S3.9. Ecological traits for each of the avian host species sampled from the Shola Sky Island bird community in the Western Ghats during 2011-2013; used for calculating ecological distance matrices in the multiple regressions on distance matrices (MRM) analysis.

Scientific Name	**Roosting**	**Foraging strata**	**Habitat**	**Minimum Elevation (m)**	**Maximum Elevation (m)**	**Elevation range (m)**
Alcippe poioicephala	Social	Middle	Forest	60	1700	1640
Anthus nilghiriensis	Non-social	Low	Grassland	1900	2500	600
Anthus rufulus	Non-social	Low	Grassland	0	2500	2500
Columba elphinstonii	Social	High	Forest	600	2500	1900
Culicicapa ceylonensis	Non-social	Middle	Forest	700	2500	1800
Cyornis pallipes	Non-social	Middle	Forest	60	1500	1440
Eumyias albicaudatus	Non-social	Middle	Forest	1000	2500	1500
Ficedula nigrorufa	Non-social	Middle	Forest	1300	2500	1200
Garrulax delesserti	Social	Low	Forest	300	1800	1500
Hypsipetes leucocephalus	Social	High	Forest	900	2400	1500
Iole indica	Social	Middle	Forest	60	1600	1540
Montecincla cachinnans	Social	Middle	Forest	1400	2500	1100
Montecincla fairbanki	Social	Middle	Forest	1400	2500	1100
Montecincla jerdoni	Social	Middle	Forest	1400	2500	1100
Montecincla meridionalis	Social	Middle	Forest	1400	2500	1100
Pellorneum ruficeps	Non-social	Low	Forest	60	1600	1540

Scientific Name	Roosting	Foraging strata	Habitat	Minimum Elevation (m)	Maximum Elevation (m)	Elevation range (m)
Pomatorhinus horsfieldii	Non-social	Middle	Forest	300	1600	1300
Pycnonotus jocosus	Non-social	Middle	Forest	0	2500	2500
Rhopocichla atriceps	Social	Middle	Forest	60	1600	1540
Saxicola caprata	Non-social	Low	Grassland	0	2500	2500
Schoenicola platyurus	Non-social	Low	Grassland	1200	1800	600
Sholicola albiventris	Non-social	Middle	Forest	1400	2500	1100
Sholicola ashambuensis	Non-social	Middle	Forest	1400	2500	1100
Sholicola major	Non-social	Middle	Forest	1400	2500	1100
Turdus merula	Non-social	Low	Forest	1400	2500	1100
Zoothera citrina	Non-social	Low	Forest	0	1600	1600
Zoothera dauma	Non-social	Low	Forest	1300	2400	1100
Zosterops palpebrosus	Social	Middle	Forest	700	2500	1800

Table S3.10. Results of Analysis of molecular variance (AMOVA) with host species and geographic regions corresponding to each Sky Island group as two levels in a hierarchical model. Analyses were carried out separately for *Plasmodium* spp. and *Haemoproteus* spp. Reported are the F-statistic for variance among Host species within Total ($F_{Host/Total}$), geographic regions within each host species ($F_{Region/Host}$) and sampling sites within geographic regions ($F_{Site/Region}$). Statistically significant results ($P < 0.05$) are in bold.

Parasite	**Level**	**F-statistic**	***P***
Plasmodium spp.	$F_{Host/Total}$	0.073	**0.045**
	$F_{Region/Host}$	0.208	**0.004**
	$F_{Site/Region}$	0.079	**0.007**
Haemoproteus spp.	$F_{Host/Total}$	0.688	**0.001**
	$F_{Region/Host}$	0.031	0.464
	$F_{Site/Region}$	0.113	**0.018**

Table S3.11. Results of multiple regressions on distance matrices (MRM) analysis. Models were run separately for *Plasmodium* and *Haemoproteus*. Analyses were carried out with pairwise parasite phylogenetic distance as the dependent matrix and host phylogenetic distance, host ecological distance, biogeographic gap (as a Boolean matrix), geographic distance and elevational distance as independent matrices. Statistically significant results ($P < 0.05$) are in bold.

Parasite	**Variable**	***B***	***t***	***P***
Plasmodium	Intercept	-0.190	-4.648	**0.003**
spp.	Host phylogenetic distance	0.020	0.809	0.592
	Host ecological distance	0.023	0.656	0.609
	Biogeographic gap	0.229	4.686	**0.003**
	Geographic distance	0.094	4.425	**0.002**
	Elevational distance	-0.027	-1.101	0.360
Haemoproteus	Intercept	-0.316	-63.178	0.999
spp.	Host phylogenetic distance	0.059	18.157	**0.014**
	Host ecological distance	0.164	44.794	**0.001**
	Biogeographic gap	0.017	3.114	0.558
	Geographic distance	0.001	0.389	0.909
	Elevational distance	0.053	16.614	**0.037**

Table S3.12. Nearest neighbor phylogenetic distance (D_{KN}) for each Shola Sky Island *Plasmodium spp.* lineage calculated across the global phylogenetic tree reconstructed with Shola Sky Island lineages and lineages from the MalAvi database. Significance was assessed by randomizing tip labels across the global phylogenetic tree. Statistically significant results ($P < 0.05$) are in bold.

Lineage	**D_{KN}**	***P***
P_EUMALB02	0.512	0.436
P_MSP02	0.620	0.588
P_TURMER07	1.210	0.940
P_MSP03	0.603	0.511
P_TURMER02	0.780	0.703
P_GARDEL02	0.395	0.326
P_MONCAC01	0.395	0.369
P_MSP04	0.784	0.722
P_MSP01	0.680	0.629
P_ANTRUF01	0.542	0.477
P_ZOOCIT02	0.349	0.279
P_TURMER06	0.349	0.280
P_TURMER04	0.061	**0.005**
P_TURMER05	0.061	**0.007**
P_TURMER03	0.473	0.391
P_MONFAI02	0.473	0.423
P_TURMER01	0.620	0.565
P_SAXCAP01	0.685	0.600
All lineages	0.533	0.272

Table S3.13. Nearest neighbor phylogenetic distance (D_{KN}) for each Shola Sky Island *Haemoproteus spp.* lineage calculated across the global phylogenetic tree reconstructed with Shola Sky Island lineages and lineages from the MalAvi database. Significance was assessed by randomizing tip labels across the global phylogenetic tree. Statistically significant results ($P < 0.05$) are in bold.

Lineage	**D_{KN}**	***P***
H_IOLIND01	0.136	0.072
H_PYNJOC01	0.312	0.315
H_HYPLEU01	0.170	0.123
H_HYPLEU02	0.066	**0.019**
H_HYPLEU03	0.066	**0.016**
H_MSP06	0.115	0.058
H_ZOSPAL03	0.115	0.070
H_ALCPOI06	0.062	**0.014**
H_ALCPOI04	0.062	**0.009**
H_ALCPOI05	0.081	**0.027**
H_MSP04	0.114	0.055
H_TURMER01	0.068	**0.024**
H_TURMER02	0.068	**0.019**
H_MSP01	0.090	**0.032**
H_MONCAC01	0.090	**0.032**
H_ZOSDAU01	0.124	0.067
H_TURMER03	0.037	**0.001**
H_CYOPAL01	0.062	**0.008**
H_MSP02	0.037	**0.003**
H_COLELP01	0.812	0.857
H_ZOSPAL04	0.636	0.742

Lineage	**D_{KN}**	***P***
H_SHOMAJ03	0.883	0.881
H_MONMER02	0.090	**0.040**
H_MONFAI02	0.090	**0.040**
H_MONCAC03	0.131	0.074
H_MSP07	0.136	0.083
H_POMHOR01	0.287	0.267
H_MSP05	0.287	0.251
H_MSP03	2.924	0.996
All lineages	0.281	**0.002**

3. Figures

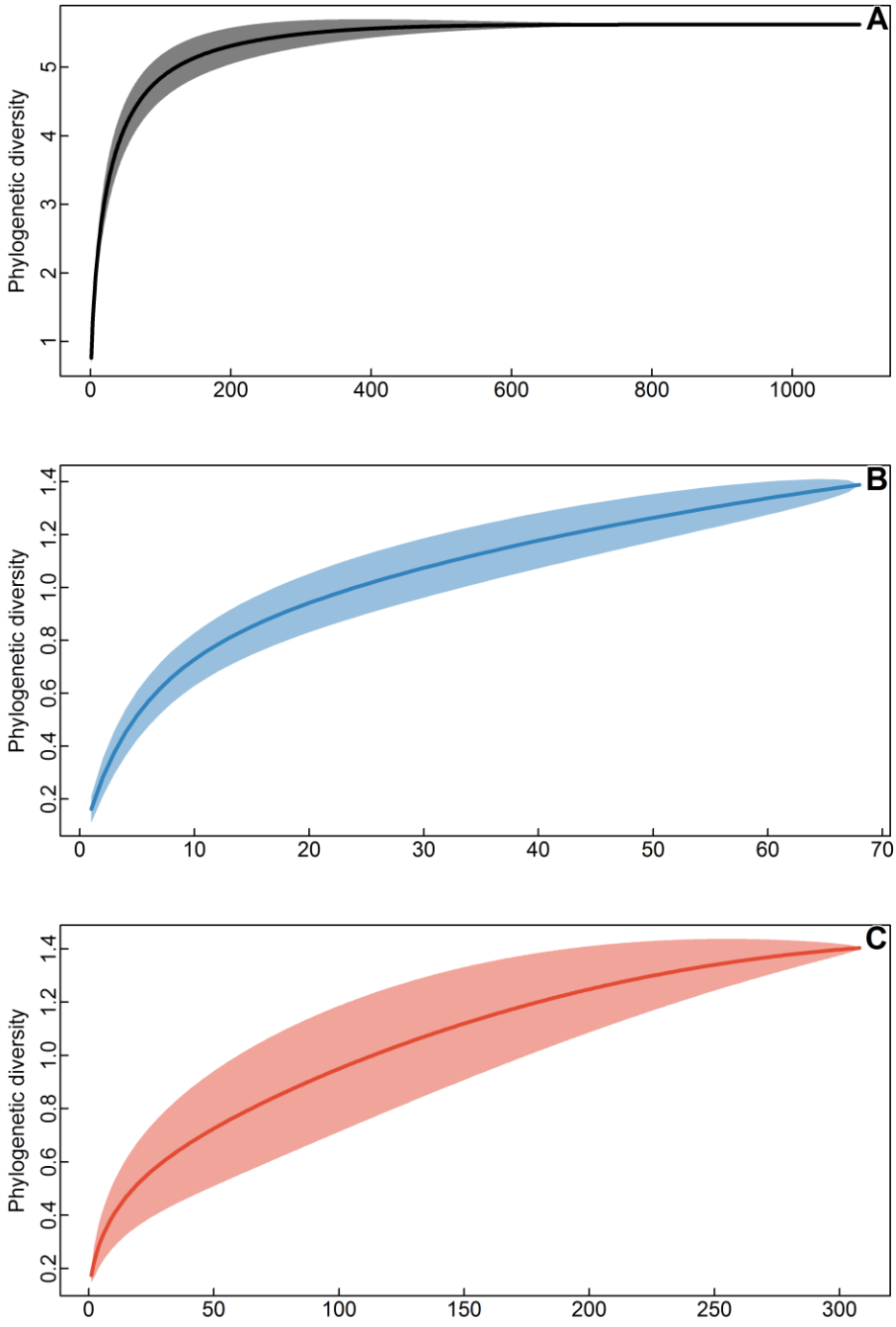


Figure S3.14. Rarefaction curves for (A) Host species; (B) *Plasmodium* spp.; (C) *Haemoproteus* spp.

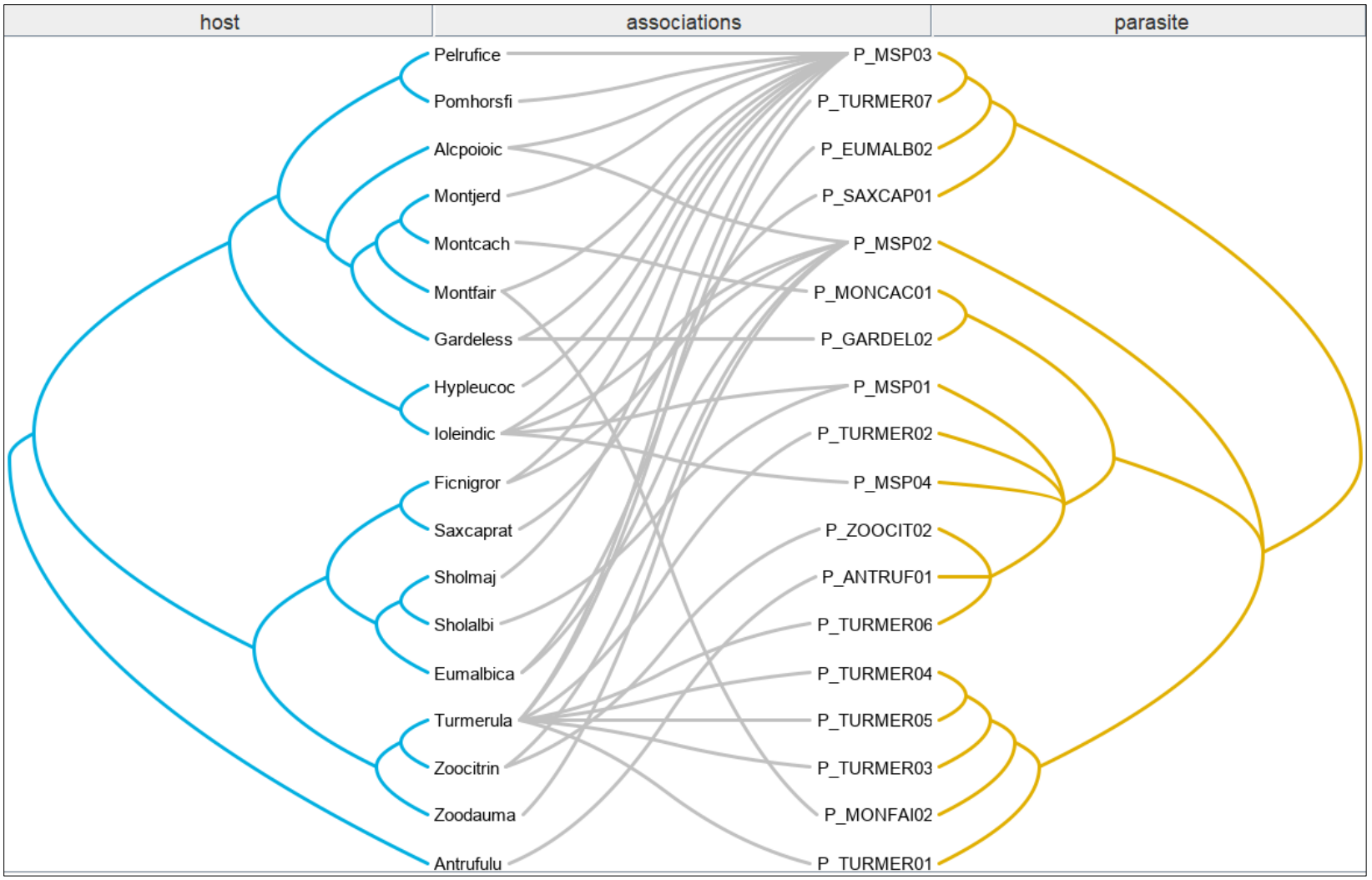


Figure S3.15. Tanglegram **c**omparing *Plasmodium spp.* phylogeny (right) and host phylogeny (left) with connecting lines indicating host–parasite associations.

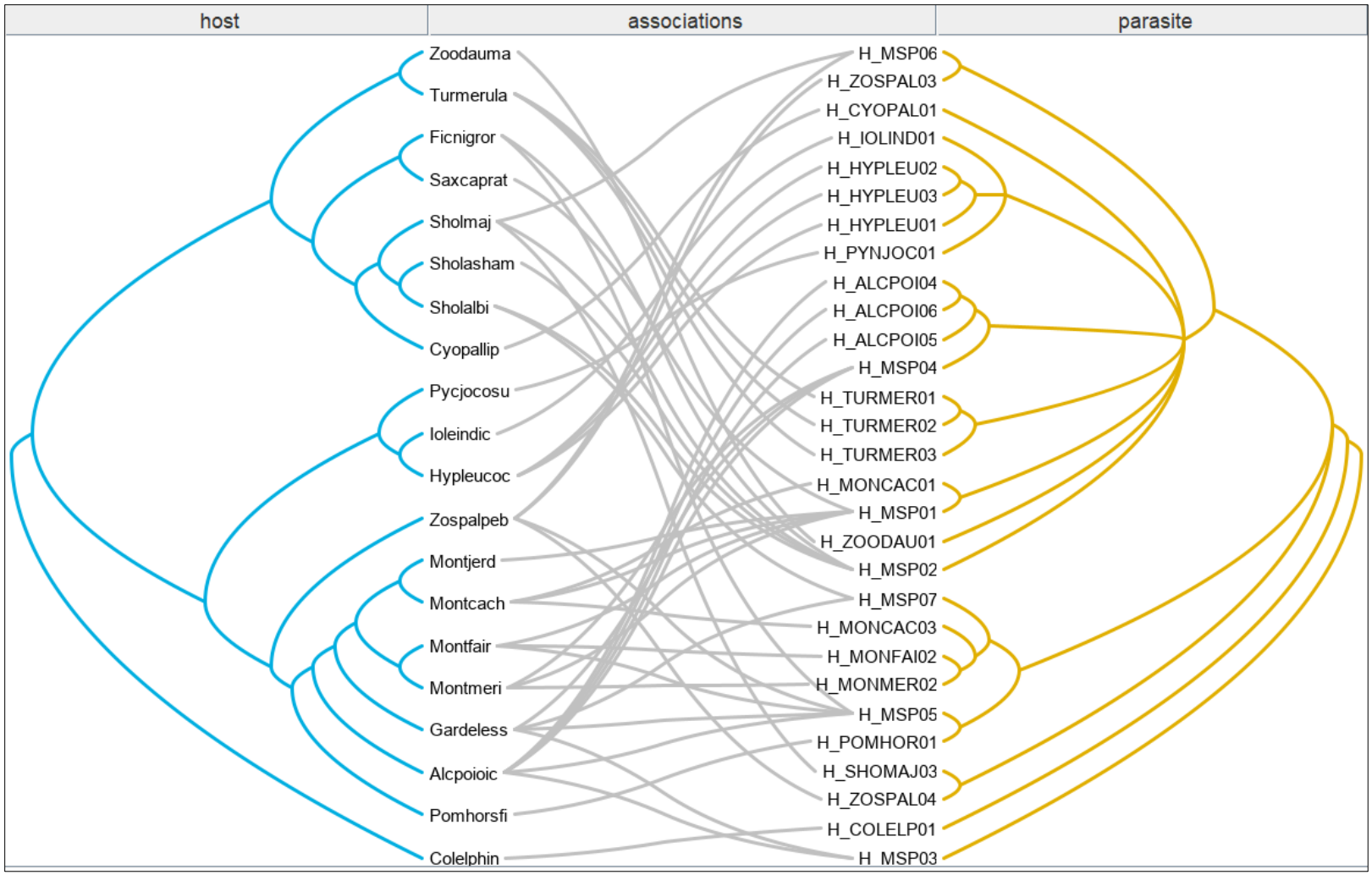


Figure S3.16. Tanglegram **c**omparing *Haemoproteus spp.* phylogeny (right) and host phylogeny (left) with connecting lines indicating host–parasite associations.

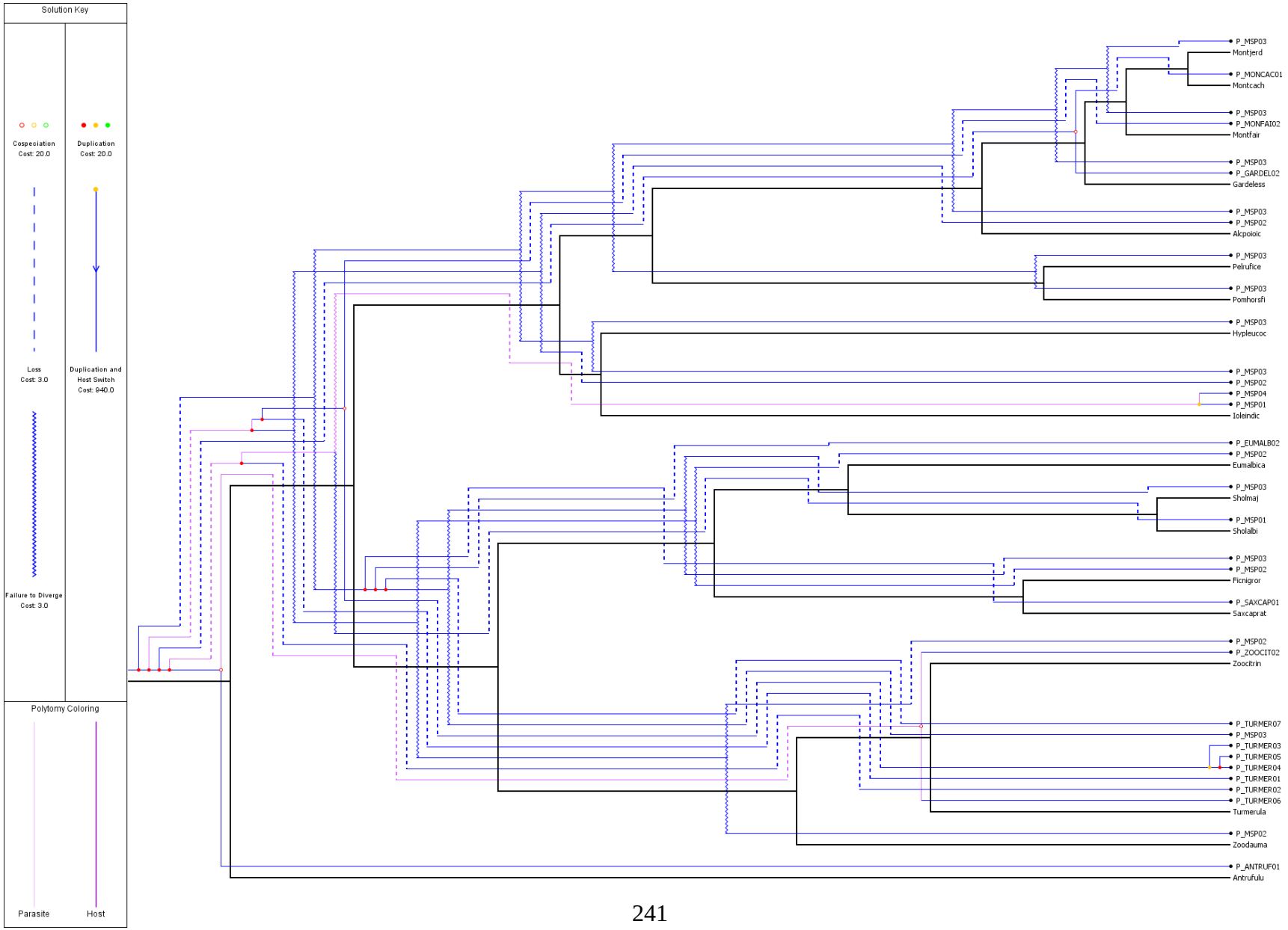

Solution Key
Cospeciation
Cost: 20.0
Duplication
Cost: 20.0
Loss
Cost: 3.0
Duplication and
Host Switch
Cost: 940.0
Failure to Diverge
Cost: 3.0
Polytomy Coloring
Parasite
Host
P_MSP03
Montjerd
P_MONCAC01
Montcach
P_MSP03
P_MONFAI02
Montfair
P_MSP03
P_GARDEL02
Gardeless
P_MSP03
P_MSP02
Alcpoioic
P_MSP03
Pelrufice
P_MSP03
Pomhorsfi
P_MSP03
Hypleucoc
P_MSP03
P_MSP02
P_MSP04
P_MSP01
Ioleindic
P_EUMALB02
P_MSP02
Eumalbica
P_MSP03
Sholmaj
P_MSP01
Sholalbi
P_MSP03
P_MSP02
Ficnigror
P_SAXCAP01
Saxcaprat
P_MSP02
P_ZOOCIT02
Zoocitrin
P_TURMER07
P_MSP03
P_TURMER03
P_TURMER05
P_TURMER04
P_TURMER01
P_TURMER02
P_TURMER06
Turmerula
P_MSP02
Zoodauma
P_ANTRUF01
Antrufulu

Figure S3.17. Least costly co-evolutionary reconstructions between *Plasmodium spp.* and host phylogenies, achieved using Jane. Black branches represent the host phylogeny and blue branches represent the parasite phylogeny. See legend for various co-evolutionary events tested using Jane.

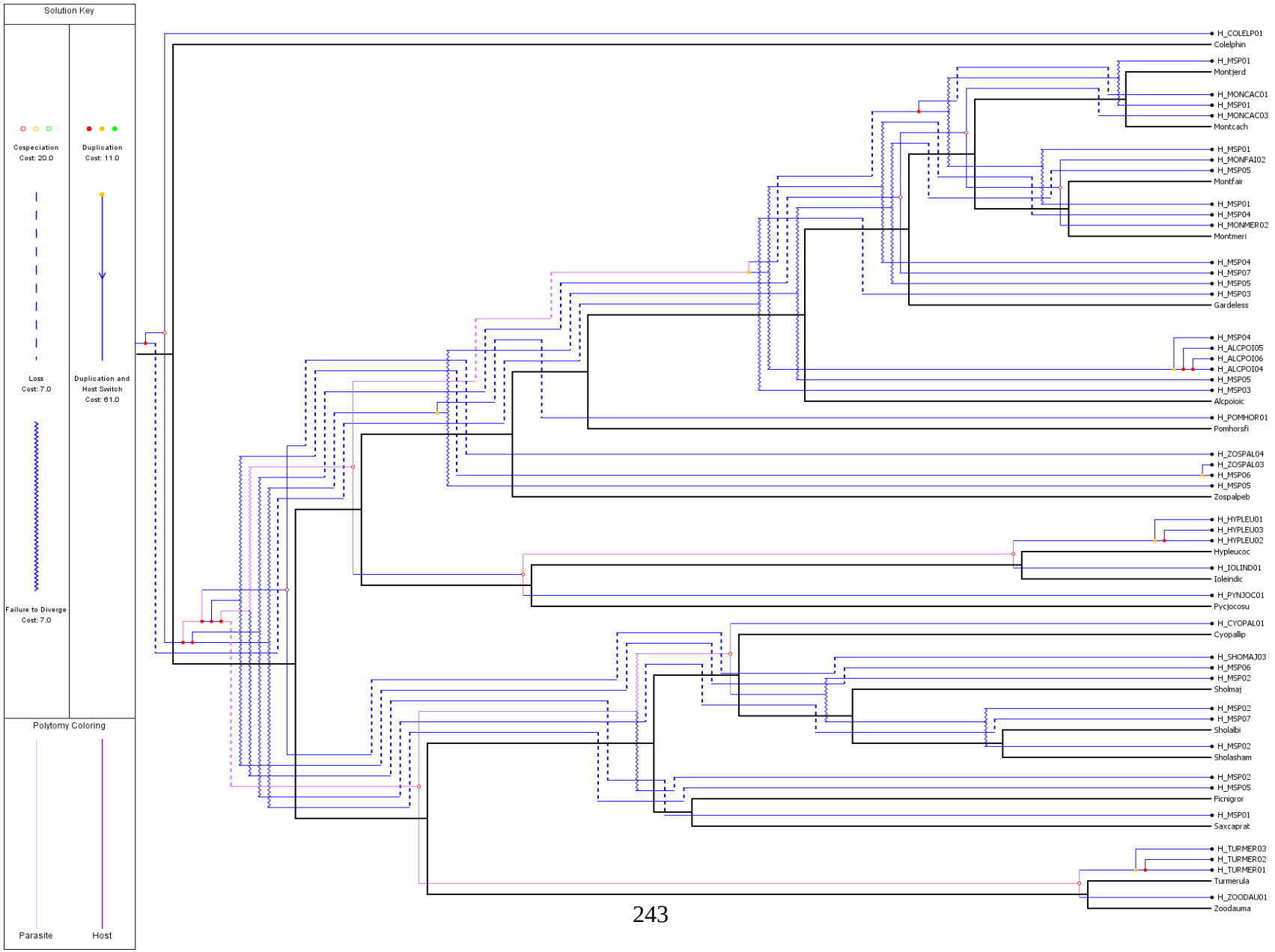

Solution Key
Cospeciation
Cost: 20.0
Duplication
Cost: 11.0
Loss
Cost: 7.0
Duplication and
Host Switch
Cost: 61.0
Failure to Diverge
Cost: 7.0
Polytomy Coloring
Parasite
Host
H_COLELP01
Colelphin
H_MSP01
Montjerd
H_MONCAC01
H_MSP01
H_MONCAC03
Montcach
H_MSP01
H_MONFAI02
H_MSP05
Montfair
H_MSP01
H_MSP04
H_MONMER02
Montmeri
H_MSP04
H_MSP07
H_MSP05
H_MSP03
Gardeless
H_MSP04
H_ALCPOI05
H_ALCPOI06
H_ALCPOI04
H_MSP05
H_MSP03
Alcpoioic
H_POMHOR01
Pomhorsfi
H_ZOSPAL04
H_ZOSPAL03
H_MSP06
H_MSP05
Zospalpeb
H_HYPLEU01
H_HYPLEU03
H_HYPLEU02
Hypleucoc
H_IOLIND01
Ioleindic
H_PYNJOC01
Pycjocosu
H_CYOPAL01
Cyopallip
H_SHOMAJ03
H_MSP06
H_MSP02
Sholmaj
H_MSP02
H_MSP07
Sholalbi
H_MSP02
Sholasham
H_MSP02
H_MSP05
Ficnigror
H_MSP01
Saxcaprat
H_TURMER03
H_TURMER02
H_TURMER01
Turmerula
H_ZOODAU01
Zoodauma

Figure S3.18. Least costly co-evolutionary reconstructions between *Haemoproteus spp.* and host phylogenies, achieved using Jane. Black branches represent the host phylogeny and blue branches the parasite phylogeny. See legend for various co-evolutionary events tested using Jane.

APPENDIX B

ELECTRONIC SUPPLEMENTARY MATERIAL FOR CHAPTER 4

1. Tables

Table S4.1. Ecological traits for each avian host species sampled from the Shola Sky Island bird community in the Western Ghats during 2011-2013. Reported are the common and scientific names of each species sampled, sample size (N_{TOT}), number of individuals infected with haemosporidian parasites (N_{INF}), proportion of infected individuals for *Plasmodium* spp. (P) and *Haemoproteus* spp. (H). The ecological factors reported include: roosting behaviour (Social or Non-social), feeding strata (High or Low), habitat type (Forest or Grassland), levels of sexual dimorphism (High or Low), phylogeographic connectivity of the species, minimum elevation at which the species has been reported.

Scientific Name	**Common Name**	**N_{TOT}**	**N_{INF}**	**Prevalence %**		**Sociality**	**Feed. strata**	**Habitat**	**SDM**	**Connectivity**	**Min.Elev (m)**
				P	**H**						
Alcippe poioicephala	Brown cheeked fulvetta	57	25	5.26	33.33	Social	High	Forest	Low	Breaks	60
Anthus nilghiriensis	Nilgiri pipit	16	0	0	0	Non-social	Low	Grassland	Low	Breaks	1900

Scientific Name	Common Name	N_{TOT}	N_{INF}	Prevalence %		Sociality	Feed. strata	Habitat	SDM	Connectivity	Min.Elev (m)
				P	H						
Anthus rufulus	Paddyfield pipit	5	1	20	0	Non-social	Low	Grassland	Low	No Breaks	0
Columba elphinstonii	Nilgiri wood pigeon	6	2	0	33.33	Social	High	Forest	Low	No Breaks	600
Culicicapa ceylonensis	Grey- headed flycatcher	29	0	0	0	Non-social	High	Forest	Low	No Breaks	700
Cyornis pallipes	White bellied blue flycatcher	17	5	0	29.41	Non-social	High	Forest	High	No Breaks	60
Eumyias albicaudatus	Nilgiri flycatcher	43	3	4.65	0	Non-social	High	Forest	High	Breaks	1000
Ficedula nigrorufa	Black & orange flycatcher	106	11	1.89	7.55	Non-social	High	Forest	High	No Breaks	1300
Garrulax delesserti	Wayanad laughingthrush	38	30	13.16	44.74	Social	Low	Forest	Low	No Breaks	300

Scientific Name	Common Name	N_{TOT}	N_{INF}	Prevalence %		Sociality	Feed. strata	Habitat	SDM	Connectivity	Min.Elev (m)
				P	H						
Hypsipetes leucocephalus	Black bulbul	58	45	12.07	51.72	Social	High	Forest	Low	No Breaks	900
Iole indica	Yellow browed bulbul	25	12	16	12	Social	High	Forest	Low	Breaks	60
Montecincla cachinnans	Nilgiri chilappan	32	19	3.13	46.88	Social	High	Forest	Low	No Breaks	1400
Montecincla fairbanki	Palani chilappan	102	71	2.94	52.94	Social	High	Forest	Low	No Breaks	1400
Montecincla jerdoni	Banasura chilappan	21	11	4.76	47.62	Social	High	Forest	Low	No Breaks	1400
Montecincla meridionalis	Ashambu chilappan	36	19	2.78	44.44	Social	High	Forest	Low	No Breaks	1400
Pellorneum ruficeps	Puff- throated babbler	27	1	3.7	0	Non-social	Low	Forest	Low	No Breaks	60

Scientific Name	Common Name	N_{TOT}	N_{INF}	Prevalence %		Sociality	Feed. strata	Habitat	SDM	Connectivity	Min.Elev (m)
				P	H						
Pomatorhinus horsfieldii	Scimitar babbler	37	8	2.7	13.51	Non-social	High	Forest	Low	Breaks	300
Pycnonotus jocosus	Red-whiskered bulbul	26	1	0	3.85	Non-social	High	Forest	Low	No Breaks	0
Rhopocichla atriceps	Dark- fronted babbler	34	0	0	0	Social	High	Forest	Low	Breaks	60
Saxicola caprata	Pied bushchat	17	2	5.88	5.88	Non-social	Low	Grassland	High	No Breaks	0
Schoenicola platyurus	Broad- tailed grassbird	7	0	0	0	Non-social	Low	Grassland	Low	No Breaks	1200
Sholicola albiventris	White bellied shortwing	100	34	1	32	Non-social	High	Forest	Low	No Breaks	1400
Sholicola ashambuensis	Ashambu shortwing	21	2	0	9.52	Non-social	High	Forest	Low	Breaks	1400

Scientific Name	Common Name	N_{TOT}	N_{INF}	Prevalence %		Sociality	Feed. strata	Habitat	SDM	Connectivity	Min.Elev (m)
				P	H						
Sholicola major	Rufous bellied shortwing	86	19	1.16	20.93	Non-social	High	Forest	Low	No Breaks	1400
Turdus merula	Blackbird	86	56	29.07	6.98	Non-social	Low	Forest	High	Breaks	1400
Zoothera citrina	Orange-headed ground thrush	10	5	30	0	Non-social	Low	Forest	High	No Breaks	0
Zoothera dauma	Scaly thrush	17	11	0	17.65	Non-social	Low	Forest	Low	No Breaks	1300
Zosterops palpebrosus	Oriental white eye	118	97	0.85	77.12	Social	High	Forest	Low	Breaks	700

Table S4.2: Detailed methods for the calculation of specific ecological and morphological variables used in the Bayesian phylogenetic mixed effect model (MCMCglmm analysis).

Variable	**Methodological details**
Species Level Analysis	
Foraging strata	We classified birds into two categories based on foraging strata: Low (ground foraging) and High (understory to mid-level foraging). Foraging strata data was based on Somasundaram et al. (2008) and Wilman et al. (2014), with the former being given precedence as it reported data specific for the Western Ghats
Roosting Behavior	Categorized into social and non-social based on observations by VVR/CKV
Habitat Type	Habitat preference was classified into two categories: Forest (species preferring evergreen, semi-evergreen, moist-deciduous, dry-deciduous, scrub habitat) or Grassland (open country, grassland) based on Ali and Ripley 1987 and observations by VVR/CKV

Minimum Elevation	We used bird distribution data to estimate the Minimum elevational distribution extent of the bird species. The distribution data were collected by collating primary and secondary data, since Indian bird sighting records are not in any database: (i) Primary data: We included sighting records of study species from over a decade's research in this area including a structured two-year survey by CKV [Sasikumar 2012], another two-year survey by VVR [Robin 2002, Robin 2006] and many field trips by VVR/CKV; (ii) Secondary data: We obtained secondary data from museum records from ORNIS database and also queried checklists and sighting records of three popular birdwatchers e-groups in south India (Bangalore bngbirds -2527 members, Kerala Birder - 918 members and Tamil Birds - 609 members). In cases where exact GPS locations were not indicated in the emails, the locations were assigned on Google Earth in what appeared to be the most appropriate nearby habitat (forest or grassland). These were subsequently extracted to a GIS platform (QGIS Lisboa ver 1.8). Museum collection locations were plotted on Google Earth only when they were unambiguous. We also used the results from a previous search (66) of three search engines (1945 to 2012) - Science Citation Index's Web of Science (Thomson Reuters, New York, USA), Biological Abstracts (Thomson Reuters, New York, USA) and Google Scholar to collect literature on the study species. We also added information from reports, books, theses and other grey literature using a snowball method (e.g. Nandini & Mudappa 2010).

Connectivity	Connectivity was classified into two categories: Breaks or No breaks depending on the presence or absence of phylogenetic structure within Western Ghats based on Robin et al. 2015.
Sexual dimorphism	Sexual dimorphism was classified into two categories: No (species with no morphological differences between sexes) or Yes (species with some morphological differences between sexes). Sexual dimorphism data was based on Ali and Ripley (1987).
Individual Level Analysis	
Std. Tarsus	Tarsus measurements were taken with Mitutoyo ABS Digimatic Caliper (Mitutoyo Corp Japan) with accuracy of 0.02 mm. Three measurements were taken each from left and right leg to account for measurement error (Lougheed et al 1991). Std. Tarsus was calculated as the average of all tarsus measurements for each bird standardized by a z-transform within each species (i.e. by subtracting the species mean tarsus measurement from the average measurement of the bird and dividing by the species standard deviation of tarsus measurements). Thus, a unit increase in Std. Tarsus indicates a one SD increase in tarsus length over mean tarsus length for the species.
Std. Wing	Wing measurements were taken with a wing rule (wing15econ Avinet Inc., New York, USA) that had a flush stop and calibration from both directions. Three measurements were taken each from left and right wing to account for measurement error (Lougheed et al 1991). Std. Wing was calculated as the average of all wing measurements for each bird standardized by a z-transform within each species (as described above).

Std. FA_{Tarsus}	To estimate the magnitude of fluctuating asymmetry in tarsal measurements, we used the mixed-effects regression model approach analyzed by restricted maximum-likelihood (REML) as proposed by van Dongen et al., 2001). Briefly, we first generate a variable Side (-1 and 1 for left and right tarsusl measurements, respectively). The fixed effects part of the model included Species, Trapping Site and Side, and thus measures the average (i.e. intercept) values of the measurement for each species and site, and estimates the level of directional asymmetry (fixed intercept of the Side variable). The random effects structure included a random intercept for each individual (measuring deviation of the individual from the fixed intercept; see above) and random slope for Side within Individual. The random Side slope measures the magnitude of FA (FA_{Tarsus}) within each individual (after controlling for other factors; i.e. individual-, species- and site-specific differences in size and magnitude of directional asymmetry). FA estimates were standardized by z-transform within each species (as described above).

Std. Body Condition Index	To estimate body condition (CI), we used the scaled mass index (SMI) as proposed by Peig and Green (2009). This index standardizes body mass to a specific fixed value of a linear body measurement based on the scaling relationship between mass and length using the equation: $\widehat{M} = M_i \left(\frac{L_0}{L_i}\right)^{b_{SMA}}$ where $\widehat{M}$ is the scaled body mass, M_i and L_i are the body weight and linear body measurement of individual i, respectively, b_{SMA} is the scaling exponent estimated by the standardized major axis (SMA) regression of M on L (log-log scale); L_0 is an arbitrary value of L (e.g., the arithmetic mean value for the study species/population). To estimate b_{SMA}, we conducted a species-wise linear regression of log-body weight on log-wing length using a type 2 (standaradized major axis regression; SMA) regression. We chose wing measurement as a linear body measurement to scale body weight because average wing length (three each from left and right wing) was most strongly correlated with body weight on a log-log scale (Pearson correlation, r = .80, p <0.001); other variable tested was average tarsal length (three each from left and right tarsus). The regression slope values are given in Table S4.5, which were used as a measure of b_{SMA}. We used average wing measurement for each species as L_0. Finally, we calculated SMI ($\widehat{M}$) as individual body weight × (average wing measurement for each species/average individual wing length) ^ b_{SMA}. Body CI measurements were standardized by z-transform within each species (as described above).

Table S4.3. Summary of results for Bayesian phylogenetic mixed models (MCMCglmm analysis) with parasite infection status as the response variable and various host ecological and morphological traits as the predictor variables. Models were run separately for *Plasmodium* and *Haemoproteus*. For each model we report the posterior mean, the 95% credible interval and the p-values. Significant (< 0.05) p-values are in bold.

Plasmodium spp.

Species-level	**Full model with all predictor variables**			**Reduced model with significant effects**		
	Posterior Mode[§]	Lower-95% CI	Upper-95% CI	Posterior Mode[§]	Lower-95% CI	Upper-95% CI
Fixed effects						
(Intercept)	**-6.129**	-10.171	-1.327	**-9.744**	-2.466	-9.744
ECO_feedStrata02.high	**-3.768**	-6.005	-1.348	**-5.074**	-1.454	-5.074
ECO_socialRoost02.social	**2.158**	-0.081	4.380	**0.306**	5.063	0.306
ECO_sdm02.yes	**2.727**	0.437	4.792	**0.837**	5.196	0.837
ECO_habitat02.grassland	-2.570	-6.657	0.994	x	x	x
ECO_connect02.breaks	1.096	-0.293	2.629	x	x	x
ECO_SppWt.div10	0.066	-0.163	0.274	x	x	x

ECO_minElev.div100	-0.005	-0.135	0.127	x	x	x
Random effects						
Species	3.935	0.421	8.573	1.296	8.953	1.296
Location	0.709	0.000	1.944	0.000	1.838	0.000
Individual-level						
(Intercept)	**-6.244**	-11.130	-1.227	x	x	x
IND_Std.Wing	-0.086	-0.733	0.454	x	x	x
IND_Std.T	-0.202	-0.832	0.444	x	x	x
IND_Std.FA.Tarsus	-0.099	-0.689	0.466	x	x	x
IND_Std.BodyCI	-0.071	-0.641	0.521	x	x	x
Random effects						
Species	10.573	5.171	17.643	x	x	x
Location	1.086	0.000	2.808	x	x	x
Haemoproteus spp.						
Species-level	**Full model with all predictor variables**			**Reduced model with significant effects**		

	Posterior Mode§	Lower-95% CI	Upper-95% CI	Posterior Mode§	Lower-95% CI	Upper-95% CI
Fixed effects						
(Intercept)	-8.972	-15.857	-1.297	-12.167	-3.554	-12.167
ECO_feedStrata02.high	0.579	-2.306	3.655	x	x	x
ECO_socialRoost02.social	5.386	2.611	8.175	3.291	8.238	3.291
ECO_sdm02.yes	-0.372	-3.214	2.561	x	x	x
ECO_habitat02.grassland	-2.187	-7.815	3.134	x	x	x
ECO_connect02.breaks	-0.930	-2.856	1.216	x	x	x
ECO_SppWt.div10	0.096	-0.156	0.366	x	x	x
ECO_minElev.div100	0.132	-0.040	0.298	0.007	0.319	0.007
Random effects						
Species	11.517	5.012	18.983	4.373	16.725	4.373
Location	1.633	0.455	3.097	0.294	3.063	0.294
Individual-level						
(Intercept)	-5.199	-11.691	0.793	-5.128	-11.959	1.302

IND_Std.Wing	0.227	-0.204	0.628	x	x	x
IND_Std.T	-0.382	-0.817	0.004	x	x	x
IND_Std.FA.Tarsus	0.196	-0.168	0.547	x	x	x
IND_Std.BodyCI2	**0.715**	0.256	1.127	**0.610**	0.206	0.968
Random effects						
Species	17.641	9.686	26.110	17.596	10.210	26.060
Location	0.445	0.000	1.374	0.487	0.000	1.383

§ Bold values indicate bayesian pMCMC Significance <0.05

Table S4.4. Phylogenetic signal or lambda (k) estimates from MCMCglmm for full and reduced models for *Plasmodium* and *Haemoproteus*, calculated as the proportion of total variance attributed to phylogenetic variance. Reported are the posterior means and 95% credible intervals.

Parasite Species	Model Type	Lambda (k)	Lower CI	Upper CI
Plasmodium	Full.model	0.234	0.045	0.433
Plasmodium	Reduced.model	0.276	0.115	0.449
Haemoproteus	Full.model	0.509	0.304	0.612
Haemoproteus	Reduced.model	0.479	0.322	0.628

Table S4.5. Standardized major axis (SMA) regression of body weight (log) on wing length (log) to estimate b_{SMA} used for calculating body condition index. Reported are the scientific names of each host species, sample size (N), Slope of the fitted standardized major axis, lower and upper confidence intervals (CI) and correlation coefficient (R^2).

Species	**N**	**Slope**	**Lower CI**	**Upper CI**	**R^2**
Alcippe poioicephala	53	2.385	1.748	3.255	0.061
Anthus nilghiriensis	12	2.134	1.198	3.801	0.308
Anthus rufulus	3	2.467	0.646	9.416	0.961
Culicicapa ceylonensis	25	2.100	1.388	3.176	0.080
Cyornis pallipes	17	1.528	0.880	2.654	0.023
Eumyias albicaudatus	40	1.848	1.307	2.615	0.000
Ficedula nigrorufa	89	1.872	1.498	2.341	0.022
Garrulax delesserti	27	1.856	1.327	2.595	0.263
Hypsipetes leucocephalus	53	1.449	1.089	1.927	0.181
Iole indica	21	2.009	1.448	2.785	0.697
Montecincla cachinnans	27	3.229	2.194	4.752	0.159
Montecincla fairbanki	84	1.879	1.510	2.339	0.039

Montecincla jerdoni	21	2.391	1.568	3.647	0.136
Montecincla meridionalis	33	2.041	1.401	2.975	0.021
Pellorneum ruficeps	23	2.199	1.462	3.308	0.198
Pomatorhinus horsfieldii	31	3.487	2.435	4.995	0.154
Pycnonotus jocosus	22	3.662	2.346	5.714	0.077
Rhopocichla atriceps	33	2.917	2.003	4.248	0.025
Saxicola caprata	15	3.467	2.203	5.457	0.137
Schoenicola platyurus	7	0.927	0.433	1.984	0.032
Sholicola albiventris	64	1.970	1.539	2.521	0.021
Sholicola ashambuensis	11	2.350	1.424	3.880	0.477
Sholicola major	79	1.643	1.298	2.078	0.042
Turdus merula	80	-1.672	-2.081	-1.342	0.044
Zoothera citrina	6	6.963	2.934	16.527	0.106
Zoothera dauma	16	-4.687	-8.525	-2.577	0.007
Zosterops palpebrosus	99	3.551	2.841	4.438	0.024

APPENDIX C

ELECTRONIC SUPPLEMENTARY MATERIAL FOR CHAPTER 5

1. Tables

Table S5.1. Detailed list of samples used for target sequence capture with malaria UCE probe set.

Sample	Parasite genus	Host organism	Source Type	Concentration (ng/uL)	Source
Clinical strains					
Plasmodium berghei -- ANKA	Plasmodium	*Mus_musculus*	Mouse blood	23.03	BEI Resources
Plasmodium chabaudi chabaudi -- AS	Plasmodium	*Mus_musculus*	Mouse blood	64.4	BEI Resources
Plasmodium yoelii -- 17XA	Plasmodium	*Mus_musculus*	Mouse blood	87.2	BEI Resources
Plasmodium gallinaceum -- 8A	Plasmodium	*Gallus_gallus*	Chicken blood	62.7	BEI Resources
Plasmodium relictum	Plasmodium	*Gallus_gallus*	Bird Blood	84.2	ATCC
Plasmodium falciparum, Strain 3D7A	Plasmodium	*Homo_sapiens*	Genomic DNA	10	BEI Resources
Plasmodium cynomolgi, Strain Bastianellii	Plasmodium	*Macaca mulatta*	Genomic DNA	10	BEI Resources
Plasmodium knowlesi, Strain H	Plasmodium	*Macaca mulatta*	Genomic DNA	50	NIH

Sample	Parasite genus	Host organism	Source Type	Concentration (ng/uL)	Source
Field strains					
DH 14	Haemoproteus	*Eudocimus albus*	Genomic DNA	6.31	Yabsley Lab
DH 18	Haemoproteus	*Eudocimus albus*	Genomic DNA	0.13	Yabsley Lab
DH 30	Haemoproteus	*Eudocimus albus*	Genomic DNA	8.02	Yabsley Lab
IC 10	Haemoproteus	*Eudocimus albus*	Genomic DNA	1.87	Yabsley Lab
IC 13	Haemoproteus	*Eudocimus albus*	Genomic DNA	3.73	Yabsley Lab
IC38	Haemoproteus	*Eudocimus albus*	Genomic DNA	0.34	Yabsley Lab
DB 08	Haemoproteus	*Eudocimus albus*	Genomic DNA	9.31	Yabsley Lab
DB 09	Haemoproteus	*Eudocimus albus*	Genomic DNA	1.77	Yabsley Lab
DB 10	Haemoproteus	*Eudocimus albus*	Genomic DNA	5.09	Yabsley Lab
LCS2	Haemoproteus	*Eudocimus albus*	Genomic DNA	5.85	Yabsley Lab
GP 05	Haemoproteus	*Eudocimus albus*	Genomic DNA	0.56	Yabsley Lab
GP 29	Haemoproteus	*Eudocimus albus*	Genomic DNA	0.106	Yabsley Lab
JB 12	Haemoproteus	*Eudocimus albus*	Genomic DNA	0.19	Yabsley Lab

Sample	Parasite genus	Host organism	Source Type	Concentration (ng/uL)	Source
30465	Haemoproteus	*Phoenicopterus ruber*	Genomic DNA	0.09	
TV11	Haemoproteus	*Cathartes aura*	Genomic DNA	0.15	Yabsley Lab
Z17-0358	Leucocytozoon	*Bubo virginianus*	Genomic DNA	>60	Yabsley Lab
Z16-466	Leucocytozoon	*Bubo virginianus*	Genomic DNA	>60	Yabsley Lab
GHOW-D359	Leucocytozoon	*Bubo virginianus*	Genomic DNA	3.91	
IDL18-1609	Plasmodium	*Spheniscidae spp.*	Genomic DNA	>60	Yabsley Lab
PFA01	Plasmodium	*Polihierax semitorquatus*	Genomic DNA	1.16	Yabsley Lab
FLA01	Plasmodium	*Phoenicopterus ruber*	Genomic DNA	0.18	Yabsley Lab
16N-1030	Mixed infection	*Bubo virginianus*	Genomic DNA	>60	Yabsley Lab

Table S5.2. Summary statistics of all samples used for target sequence capture with malaria UCE probe set. Reported are the sample IDs (with parasite genus, ID, pool and run no. separated by underscores), treatments used for obtaining two datasets, number of raw paired reads, total number of cleaned reads after adapter removal and quality trimming, percentage of reads mapped to parasite genomes, number of contigs assembled from mapped reads, total bp, mean, CI, minimum and maximum contig length, number and percentage of UCE loci recovered from multiple contigs and number of UCE loci removed. Highlighted Sample IDs represent the samples that had greater than 100 UCE loci recovered (except for 4 samples with <50 UCE loci) and used in the final phylogenetic tree.

Sample ID	Treatment	Raw	bwa-mapping QC		mapped-reads-abyss-assembly-QC					UCE-Loci-stats		Loci-removed	
		Paired reads	Total cleaned reads	% Aligned	Contigs	Total kbp	Mean Len.	Min Len.	Max Len.	UCE Loci Found	% Loci found	Multi contig hits	Multi UCE hits
P_kno_den_r3	dil-capture	4.6	8.8	98.90%	706	156.06	221.05	104	1077	250	35.41	12	22
P_cha_den_r3	dil-capture	15.6	30.8	96.00%	306	94.42	308.56	118	1278	249	81.37	6	42
P_gal_en	post-capture	20.4	36.3	27.70%	548	169.22	308.79	109	1469	249	45.44	7	46
P_yoe_den_r3	dil-capture	4	7.9	83.20%	563	128.00	227.36	111	1228	247	43.87	9	18

Sample ID	Treatment	Raw	bwa-mapping QC		mapped-reads-abyss-assembly-QC					UCE-Loci-stats		Loci-removed	
		Paired reads	Total cleaned reads	% Aligned	Contigs	Total kbp	Mean Len.	Min Len.	Max Len.	UCE Loci Found	% Loci found	Multi contig hits	Multi UCE hits
P_cyn_en	post-capture	32	58.1	76.30%	321	113.00	352.03	119	1760	243	75.7	3	60
H_JB12_5_nr2	single	479.9	941.8	12.30%	2034	312.57	153.67	101	1360	232	11.41	22	30
P_fal_unen	pre-capture	316	587.1	99.50%	35049	9900.38	282.47	101	3322	232	0.66	29	33
P_kno_en	post-capture	172.1	294.6	98.90%	394	191.13	485.10	103	2069	230	58.38	13	62
P_fal_den_r3	dil-capture	33.2	65.26	99.92%	310	108.07	348.62	101	1782	226	72.9	7	52
P_gal_2_r1	single	1084.4	2043.4	34.40%	2530	424.59	167.82	101	1789	225	8.89	44	52
P_fal_en	post-capture	1077.1	1975.9	99.92%	303	159.32	525.81	107	2533	224	73.93	7	61
H_JB12_5_nr3	double	827.4	1639.2	48.10%	521	107.18	205.71	101	1360	223	42.8	39	33
P_PFA01_2_r3	double	494.4	973.8	71.70%	371	102.32	275.80	101	1392	223	60.11	16	49

Sample ID	Treatment	Raw	bwa-mapping QC		mapped-reads-abyss-assembly-QC					UCE-Loci-stats		Loci-removed	
		Paired reads	Total cleaned reads	% Aligned	Contigs	Total kbp	Mean Len.	Min Len.	Max Len.	UCE Loci Found	% Loci found	Multi contig hits	Multi UCE hits
P_yoe_en	post-capture	124.3	235.5	91.40%	292	118.45	405.66	108	1747	222	76.03	6	59
P_cha_en	post-capture	~~465.8~~	898.1	98.00%	298	134.48	451.29	102	1807	220	73.83	7	64
P_rel_2_r1	single	1003.5	1898.3	27.80%	2861	449.98	157.28	101	1784	219	7.65	32	54
H_DH18_5_nr2	single	509.4	1005.5	25.10%	2532	380.98	150.46	101	1433	217	8.57	55	32
P_ber_en	post-capture	283	530.4	94.50%	305	128.50	421.31	106	1892	216	70.82	7	62
P_ber_den_r3	dil-capture	9	17.7	90.90%	280	67.53	241.19	102	1243	210	75	3	31
P_FLA01_4_r2	single	5063	9932.6	17.90%	26520	3496.27	131.84	101	1218	188	0.71	146	27
H_DH18_5_nr3	double	1844.3	3656.8	57.00%	1110	174.36	157.08	101	780	187	16.85	119	23
P_rel_2_r3	double	3150.2	6237.8	86.00%	1110	192.40	173.33	101	1448	185	16.67	128	37
L_Z16_2_r3	double	13.5	22.8	43.40%	456	83.84	183.85	106	520	165	36.18	61	15

Sample ID	Treatment	Raw	bwa-mapping QC		mapped-reads-abyss-assembly-QC					UCE-Loci-stats		Loci-removed	
		Paired reads	Total cleaned reads	% Aligned	Contigs	Total kbp	Mean Len.	Min Len.	Max Len.	UCE Loci Found	% Loci found	Multi contig hits	Multi UCE hits
P_cha_unen	pre-capture	281.1	531.6	40.90%	25613	4611.24	180.04	101	1866	164	0.64	9	7
P_PFA01_5_nr3	double	245.8	483.4	6.60%	1368	224.88	164.38	101	438	161	11.77	41	5
P_rel_en	post-capture	9.5	17.1	18.40%	373	70.91	190.11	102	762	161	43.16	5	17
P_gal_5_nr3	double	422.9	831.5	19.80%	1026	153.84	149.94	101	904	149	14.52	68	13
H_TV11_5_nr3	double	338	663.7	14.80%	2140	335.15	156.61	101	512	148	6.92	58	4
P_rel_5_nr3	double	190.8	377.3	9.30%	1150	178.46	155.19	101	567	142	12.35	27	6
H_DB09_5_nr2	single	1138	2245.1	42.90%	3346	458.23	136.95	101	884	141	4.21	203	16
H_IC38_5_nr3	double	264.4	519.9	11.00%	1707	276.13	161.76	101	508	132	7.73	20	3
P_gal_5_nr2	single	925.6	1821.2	5.50%	3367	473.00	140.48	101	904	132	3.92	4	9
P_ber_unen	pre-capture	469	869.3	28.00%	24305	4010.68	165.01	101	6322	109	0.45	8	2
M_16N1_2_r1	single	205	397.9	13.00%	1260	188.07	149.27	101	1053	105	8.33	77	13
M_16N1_2_r3	double	604.8	1199.8	60.10%	429	77.49	180.62	101	933	93	21.68	89	14

Sample ID	Treatment	Raw	bwa-mapping QC		mapped-reads-abyss-assembly-QC					UCE-Loci-stats		Loci-removed	
		Paired reads	Total cleaned reads	% Aligned	Contigs	Total kbp	Mean Len.	Min Len.	Max Len.	UCE Loci Found	% Loci found	Multi contig hits	Multi UCE hits
P_cyn_den_r3	dil-capture	0.9	1.8	66.30%	186	31.54	169.55	111	407	90	48.39	5	0
P_kno_unen	pre-capture	143.1	244.7	87.40%	15291	2536.97	165.91	101	2047	64	0.42	3	1
H_DH14_2_r3	double	198.6	387.9	8.20%	684	111.17	162.54	101	387	45	6.58	3	3
P_IDL18_1_r1	single	57.9	110.7	4.10%	579	96.11	166.00	101	413	40	6.91	1	0
H_GP05_3_r3	double	2611.6	5086.9	12.80%	5529	762.19	137.85	101	758	36	0.65	58	0
P_yoe_unen	pre-capture	248.1	460	20.60%	9459	1338.12	141.47	101	768	31	0.33	0	0
P_gal_den_r3	dil-capture	0.6	1.2	28.80%	65	10.06	154.72	116	238	30	46.15	2	1
H_DB10_3_r3	double	1185.4	2307.3	12.40%	2658	380.05	142.98	101	665	23	0.87	17	0
H_LCS2_3_r3	double	1731.4	3386.4	8.50%	4192	625.90	149.31	101	759	23	0.55	18	0
H_TV11_2_r1	single	1129.6	2209	11.70%	10529	1451.76	137.88	101	647	23	0.22	5	1
H_TV11_2_r3	single	643.1	1268.7	13.00%	3704	549.41	148.33	101	444	23	0.62	14	0
H_IC13_3_r3	double	2397.3	4690.2	6.50%	4483	669.99	149.45	101	639	22	0.49	10	0
H_TV11_5_nr2	single	884	1741.8	8.70%	4082	560.92	137.41	101	1030	21	0.51	2	1

Sample ID	Treatment	Raw	bwa-mapping QC		mapped-reads-abyss-assembly-QC					UCE-Loci-stats		Loci-removed	
		Paired reads	Total cleaned reads	% Aligned	Contigs	Total kbp	Mean Len.	Min Len.	Max Len.	UCE Loci Found	% Loci found	Multi contig hits	Multi UCE hits
H_IC10_3_r3	double	2267	4440.3	5.90%	3038	439.46	144.66	101	746	15	0.49	2	0
H_IC38_3_r3	double	589.7	1152.9	10.20%	1401	203.38	145.17	101	498	15	1.07	10	0
P_PFA01_5_nr2	single	791.8	1559	4.90%	2786	379.90	136.36	101	387	12	0.43	1	0
H_DB09_5_nr3	double	12143.8	24100	59.60%	80048	8817.83	110.16	101	549	10	0.01	372	72
P_rel_5_nr2	single	408.4	807.3	9.40%	2685	364.71	135.83	101	343	10	0.37	4	1
P_gal_2_r3	double	8532	16865	94.00%	107133	11878.48	110.88	101	879	9	0.01	373	82
P_rel_den_r3	dil-capture	0.4	0.8	20.10%	30	4.80	159.93	110	237	9	30	1	1
H_IC38_5_nr2	single	806.9	1587.7	8.40%	3653	504.52	138.11	101	722	7	0.19	3	0
P_cyn_unen	pre-capture	249.4	457.3	8.40%	4393	589.74	134.24	101	259	4	0.09	0	0
H_DB08_3_r3	double	817.6	1590.2	0.60%	142	21.02	148.01	104	298	3	2.11	1	0
P_rel_unen	pre-capture	345.2	626	4.90%	5185	690.47	133.17	101	568	3	0.06	0	0
H_GP29_3_r3	double	603.2	1173.2	0.50%	11	1.39	126.36	104	147	2	18.18	0	0
P_gal_unen	pre-capture	351.4	646.1	4.40%	5214	695.09	133.31	101	678	0	0	0	0

Sample ID	Treatment	Raw	bwa-mapping QC		mapped-reads-abyss-assembly-QC					UCE-Loci-stats		Loci-removed	
		Paired reads	Total cleaned reads	% Aligned	Contigs	Total kbp	Mean Len.	Min Len.	Max Len.	UCE Loci Found	% Loci found	Multi contig hits	Multi UCE hits
L_Z16_2_r2	single	14.4	10.729	8.88%	NA	NA	NA	NA	NA	NA	NA	NA	NA
L_Z17_2_r3	double	too few reads											

Table S5.3. Comparison between post-capture, diluted capture and pre-capture libraries in terms of number of total reads, percentage of reads mapped to respective parasite genomes and number of UCE loci recovered from assembled contigs of mapped reads and fold increase in the percentage of mapped reads between pre-capture and post-capture libraries.

Parasite Sample ID	Post-capture			Diluted-capture			Pre-captur			Post-capture vs pre-capture % fold increase
	K Total reads	% Reads Mapped	UCE. Loci recovered	K Total reads	% Reads Mapped	UCE Loci recovered	K Total reads	% Reads Mapped	UCE Loci recovered	
P_berghei	530.4	94.5	216	17.7	90.9	210	869.3	28.0	109	3.38
P_chaubadi	898.1	98	220	30.8	96	249	531.6	40.9	164	2.40
P_cynomolgi	58.1	76.3	243	1.8	66.3	90	457.3	8.4	4	9.08
P_gallinaceum	36.3	27.7	249	1.2	28.8	30	646.1	4.4	0	6.30
P_knowlesi	294.6	98.9	230	8.8	98.9	250	244.7	87.4	64	1.13
P_relictum	17.1	18.4	161	0.8	20.1	9	626	4.9	3	3.76
P_yoelli	235.5	91.4	222	235.5	91.4	247	460	20.6	31	4.44
P_falciparum	1975.9	99.92	224	65.26	99.92	226	587.1	99.5	232	1.00